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Nuclear secrets: no clear frontiers

WHAT is a military secret? And how does it lose its secret status? Until a few years ago, the answers to these questions were thought to be relatively straightforward — a secret was information with the word 'secret' stamped on it; it ceased being a secret when the word 'secret' was removed. But the public, or at least a portion of it, is no longer quite so trusting of military judgement and recently even the holy of holies — details on the construction of nuclear weapons — has been violated in various publications, most notably the article on hydrogen bombs by Howard Morland that has just appeared in *The Progressive*, and the long open letter to US Senator Charles Percy from Charles Hansen, a Californian computer programmer whose pastime is the study of nuclear weapons.

Publication of these documents has been accompanied by some ringing statements about press freedom. For instance, in printing the Hansen letter, the *Chicago Tribune's* Publisher Stanton Cook declared: "We are convinced it contains no secret information that would in any way jeopardise the national security. We are confident that the information was gathered from public records available to any researcher . . . The government's attempt to muzzle a private citizen and the press (is) repugnant to the First Amendment's guarantees of free speech and press . . ."

Now Mr Cook is right (or almost) on two counts. It is indeed possible to accumulate information that is in the public domain and come up with some sound conclusions concerning the design of nuclear weapons. And the revelations of the last month are certainly not going to put the United States at risk — at least in the near future. But to go on to claim freedom of speech and of the press as justification for broadcasting of nuclear weapons data could, just conceivably, be a serious misuse of these fine but not absolute principles.

Consider first the question of the accumulation of public information. There is a serious danger in the view that something

is either totally in the public domain or totally secret. There is almost no secret which cannot be deduced if a researcher is prepared to devote enough time, effort and money to the sifting and collating of evidence. The only thing the holder of secrets can do is to make it immensely difficult to extract those secrets. There is thus no great merit in the case that a secret is no longer a secret when it can be deduced from the public domain. It may still be worth keeping the price of learning the secret very high.

But, it may be argued, there is no point in keeping the secret any longer, either because the US government shouldn't be making nuclear weapons (as Mr Morland might argue) or because the US government should go on making weapons, but for other people to know how won't harm the United States (as Mr Cook might argue).

This is to act in ignorance of the Non-Proliferation Treaty. The treaty to which the United States is party, declares that nuclear-weapons states will not in any way assist, encourage or induce any non-nuclear-weapon state to acquire nuclear weapons. It is, of course, debatable whether the two documents in question do 'in any way assist', but simply because there are sincerely held views that they do, there seem powerful reasons why the narrower question of American security should yield to the broader one of global non-proliferation.

None of this should be seen as a blanket endorsement of military secrecy, much of which is preposterous and merely classifies material of low quality to protect it from critical scrutiny by outsiders. Nor should it be seen as in any way a retreat from the view that matters of nuclear policy (as opposed to hardware) are inadequately debated in public, because of the paucity of policy information available, particularly in the United Kingdom. It is simply a statement that in a difficult field where there are no clear frontiers, the case for the recent publications is not as strong as might seem at first sight. □

Overpaid humanitarians

MANY scientists in Britain speak with justifiable resentment about the tax-free salaries that employees of international organisations command. People subjected to no more privation than to live in another country (and sometimes not even to have to learn the language) are frequently paid sums which seem grotesque by continental European and US standards, let alone by those of poverty-stricken Britain.

The latest post to come to our attention is that of Chief of Public Information for an 'International Humanitarian Organisation in Geneva' (which prefers to remain anonymous).

The holder of this position will, to be sure, have to be fluent in French and English, and will have to act as spokesman for the organisation running a department of fifteen. The annual salary is round \$55,000 to \$60,000 — net.

There are highly dedicated information officers in many national humanitarian organisations around the world who count themselves lucky to receive a quarter of this sum — gross. International organisations, particularly those connected with humanitarian aims, have gravely lost their sense of direction when they offer enormous salaries. □



1979 Nobel Prizes for Medicine, Physics, and Chemistry

35th prize for inventor of EMI X-ray scanner

"I'm not the sort of person to build model planes" said Godfrey Hounsfield, co-winner of this year's Nobel prize for medicine, last week. "I've always searched for original ideas; I am absolutely opposed to doing something someone else has done."

Dr Hounsfield invented the EMI 'brain scanner' for computerised tomography — using X-rays to make two-dimensional images of slices of a patient's brain. "It came to me on a ramble" he said. The device, and its successor, the whole body scanner, brought EMI medical division a ten-fold increase in profits from 1973 to 1977, when the US market collapsed from 40 scanners a month (at £250,000 each) to just eight (see following story).

To date, Dr Hounsfield has received 35 academic awards for his work on the scanner, beginning with the MacRobert Award (the UK's major prize for engineering) in 1972, a Fellowship of the Royal Society, a CBE and many foreign degrees, and ending with the Nobel. He hopes to use his £44,000 prize to buy scientific equipment for his home "so I have something to do when I retire".

Sixty years old and a bachelor, Dr Hounsfield hasn't much time for the arts. "Science has very much a 3rd place in this country; I'd like to do something about that. The sciences are far more exciting than the arts, than fantasy".

Farming educated Hounsfield. "I was brought up in the country; I played around with farm machinery, and to prevent myself being bored I started to reason why

things work".

EMI's central research laboratories have provided Hounsfield with "a very relaxed place to do original thinking; and there's just enough pressure to produce a product. It enables one to come to a logical conclusion rather quicker than one would in academic life".

Dr Hounsfield does not know his co-winner, Allan Cormack, and says he first heard of him when he was recommended as a referee for a paper in 1972-3. Cormack had taken a mathematical approach to computerised tomography, whereas, says Hounsfield "I find I've got other tools of thinking than maths — the most important thing is a very broad understanding of the problem, not to be fogged by detail".

"In the last few years I've spent half my time on developments of the scanner, half on other things" says Hounsfield. A new version, the '7000', will cut scan time from 18 to 3s, so reducing the effects of organ movement in the body. "I've always been interested in the heart; we are working on pictures synchronised to the heart to 'arrest' the heartbeat."

Dr Hounsfield has also been working on newer, non-invasive techniques, such as nuclear magnetic resonance. This "looks promising, but isn't diagnostically useful yet. But we've a long way to go before we reach a brick wall." Further developments of the scanner involve a system directly linked to a machine for delivering therapeutic X-rays, so the surgeon can see precisely where he is working. "We're well ahead on that".



Godfrey Hounsfield: started with farm machinery

"The scanner can save money if it's used properly" says Hounsfield. "It's said the head machines can pay for themselves in 2 years" by avoiding the need for exploratory surgery or dangerous techniques like pumping air into the ventricles or heavy metals into the veins of the brain, which are the more traditional techniques for revealing defects.

Nevertheless the profits of EMI medical electronics have fallen from £14.7m in 1977 to a loss of £12.8m this year, in part because of the spectacular collapse of the US market for the scanner. And meanwhile EMI has been fighting patent rights battles with three US manufacturers of scanners: Ohio Nuclear, which is now to work under an EMI licence, and Pfizer Inc., and GEC.

Robert Walgate

● A six-month tenure at the Groote Schuur hospital in Capetown gave Allan Cormack, a native South African, first-hand knowledge of the limitations of the X-ray techniques being used. "I saw these girls

Dithering on the deadline

PROFESSOR Cormack and Dr Hounsfield became Nobel laureates after a controversy that prolonged the announcement of the prize by more than an hour. Until the last minute, it was unclear whether they or another group would share the award.

The rival nomination was reportedly for a group of three American and French scientists who have made crucial discoveries in immunogenetics. As a result of these discoveries, organ transplant have been made easier.

Exactly who these scientists are is not being revealed. But the Nobel committee, a group of 15 professors from the Karolinska Institute which goes through all the nominations and prepares a short-list, was so sure that the group would be approved that it had prepared its publicity material only on the immunogeneticists, in the usual

languages: Swedish, German, French and English.

When the committee presented its proposals to the Nobel Assembly for final decision, however, the Assembly — composed of the 64 full professors at the Karolinska Institute — argued in favour of Cormack and Hounsfield instead. After a prolonged discussion, the committee's secretary, Professor Jan Lindsten, left the deliberations to face the waiting journalists. He made the official announcement in Swedish only: the translations came later. "There was no time to check with the translators" he told *Nature* — and the foreign correspondents had to manage as best they could.

In voting for Cormack and Hounsfield, the Assembly decided to give the prize to applied instead of basic research. The relative merits of these was reportedly one of the subjects of the Assembly's discussion. The information secretary of the Royal Academy of

Sciences, Lennart Daleus, wondered afterwards how such a controversy could have arisen at the last minute. By the time the Academy's classes of physicists, chemists and economists present the full Academy with their proposals for the respective prizes, he mused, all the substantive disagreements have usually been ironed out.

Nominations for the medicine prize are invited from many sources. According to Professor Lindsten, invitations to nominate are sent to previous laureates, professors of Scandinavian faculties of medicine, and about 40 faculties of medicine in different parts of the world.

After the drama of the announcement, none of the Assembly's members was willing to talk about the controversy. The remark made by the Assembly's chairman, Professor Georg Klein, was typical of the others' tantalising restraint. "I have a lot of comments", he told *Nature*, "but I can't make them."

Wendy Barnaby

preparing all these X-ray treatments, and as a physicist, it struck me as a very rough process," Dr Cormack said. "How can you know how to treat something in the middle of the head if you do not have proper information of the effect on the radiation of the material through which it has to pass?"

The question appealed to him as a scientist. But the task of working out the answer was "mostly a mathematical one", extrapolating what physicists already knew about the attenuation of radiation passing through a homogenous material to similar calculations for inhomogenous materials.

"After publication in 1963 there was virtually no response at all, except for a few physicians, until someone came along in about 1970 to say that similar ideas had been developed by engineers at EMI," says Mr Cormack. He says that he has never met Godfrey Hounsfield, with whom he now shares the Nobel Prize, but adds that "I have heard that he is an inventive person."

Cormack's lack of involvement with the commercial development of computerised comography (CT) scanners means that he has been able to watch from the sidelines the intense debate which has taken place in the US — and which promises to be revived by the Nobel Prize award — over the use of the technology. In particular, this debate has focussed on the extent to which the federal government should support the growth of a technology whose extremely high capital costs have contributed to the rapid increase in hospital care costs in recent years.

Mr Cormack is critical of the US government's decision to place stringent restrictions on the use of CT. This move followed a report from the National Academy of Science's Institute of Medicine pointing out that in many cases, conventional X-ray techniques were much cheaper and might be just as effective (*Nature*, 12 May 1977).

"The arguments against the CT-scanner leave out a lot of things. In doing a cost-benefit analysis, for example, how much do you put in for the pain and suffering of patients for whom exploratory surgery might be avoided by using the scanner?" Mr Cormack says.

"I think the scanner has become a symbol, it is used as a whipping boy as a way of pointing out the expense of medical care; intensive care units probably cost a lot more money, but you do not usually hear about them."

At the same time, however, he admits that the cost of the basic equipment might be brought down if manufacturers were not under great pressure from physicians to improve its performance, for example through faster scans or higher resolution. "A substantial part of the costs must still be in development. If they could standardise the model, the costs would probably go down a lot," Mr Cormack says.

David Dickson



Neutral currents bring rewards

THIS is the prize that many physicists have been waiting for — not least Abdus Salam, of Imperial College, London, and the International Centre for Theoretical Physics, Trieste, and Steven Weinberg of Harvard, whose unified model of the action of the weak and electromagnetic forces has been finding corroboration in an increasing number of experiments.

But one of the severest tests of the theory — the discovery of heavy counterparts to the photon, the 'intermediate vector bosons', is yet to come, and a few will no doubt consider the prize to be premature. Experiments planned at CERN, the European centre for subnuclear physics, for 1982 are expected to provide the first direct evidence of the particles.

However the citation refers to the prediction "*inter alia*" of neutral currents, and here the theory is on firm ground. Sheldon Glashow, of MIT, played a key role in their prediction independently of the machinery of the Salam-Weinberg model, and hence his well deserved share in the prize.

The term 'neutral current' is a somewhat confusing epithet for weak interactions not involving the exchange of electric charge. For example, the familiar weak decay of atomic nuclei involves neutrons changing into protons (or *vice versa*), the excess charge being taken up by the creation of an electron and an antineutrino (or their antiparticles). This is supposed to take place by the emission and reabsorption (exchange) of a *charged* intermediate vector boson between the nucleon and electron systems. Neutral current interactions, on the other hand, involve the exchange of a neutral IVB, and so change protons into protons, electrons into electrons, and so on.

The weak force being extremely weak, the effects of these 'neutral' exchanges are completely masked by the analogous exchange of photons which creates the electromagnetic force between, say, protons and electrons. (It is this analogy, indeed, which allows the two forces to be

unified in an all embracing theory.)

The detection of neutral currents has thus proved extremely difficult, and its history has not been without its conflicts of evidence. The first observation was made with neutrinos (where electromagnetic effects are absent) at CERN; others followed, and gradually the neutrino evidence has built up into an impressive body of evidence not only for the existence of the neutral current, but for the exact form of it predicted by Glashow and incorporated by Salam and Weinberg in their (independently generated) 1967 and 1968 unified theory. More recently, a brilliant experiment with polarised electrons at the Stanford Linear Accelerator Center in California measured the interference between the electromagnetic and neutral weak interactions between electrons and protons, and also confirmed predictions.

Robert Walgate

Boron and phosphorus

Herbert C Brown of Purdue University, Indiana, and Georg Wittig of Heidelberg University share the Nobel Prize for chemistry. The citation describes their work on the application of boron and phosphorus compounds respectively to synthetic organic chemistry.

Professor Wittig, who is now over 80 but still academically active, is best known for the development of Wittig reagents, a class of phosphorus-based compounds used to form carbon-carbon double bonds at precisely predetermined places in organic molecules. They have applications in the synthesis of many compounds, including prostaglandins.

Professor Brown, on the other hand, has developed a series of very versatile organo-boron compounds which can selectively modify functional groups in organic molecules, and is also renowned for his theoretical contributions to carbo-cation chemistry. His birthplace was in the UK, but he has pursued his career in the US. □

Fellowships proposed to help US jobs problem

THE US National Science Foundation should be prepared to spend up to \$30 million a year over the next twenty years to reduce the potential impact of demographic changes on the quality of university research, according to a report published last week by the National Research Council, the research arm of the National Academy of Sciences.

Addressing a problem of increasing concern both to research scientists and to science policy makers, the report suggests that the NSF set up a new fellowship scheme to provide support for up to 250 university science faculty members for a period of five years.

In return, the scientist's university would be required to use the money which would have been used to support the scientist to recruit a new faculty member. The report says that the total cost of the programme would be about \$381 million (at 1979 prices) over a 20 year period, a sum which would represent about 3% of the NSF's annual budget. After 20 years it predicts that a steady state will have been reached with faculty recruitment balancing retirement and that federal intervention will no longer be necessary.

There are two particular aspects of current demographic changes which threaten — and in some cases are already exerting — a considerable constraint on the ability of US universities to produce high quality research, says the report: declining student enrolment and a low level of faculty retirement in a number of scientific disciplines.

The report points out that whereas in the mid-1960s higher education institutions were expanding at an average rate of 13.2%, the current rate of expansion is only about 3%, and with a rapidly falling number of 18 year olds in the population, "the prospects are very real that such enrolments will actually decline". This has inevitably led to a decline in the rate of faculty growth, it says.

An even greater impact on research, however, is likely to come from the current low retirement rate. This is partly due to the fact that universities expanded rapidly in the 1960s, after a relatively slow build up during the previous decades, and that faculty members recruited at this time are still far from retirement age — and to the impact of new legislation, which will make it illegal after 1 July 1982 for universities and colleges to establish a mandatory retirement age of less than 70.

"It is not known what proportion of faculty who under previous law would have been required to retire at age 65 will instead choose to stay on longer," the report says. It takes some pains to point out that the situation differs between disciplines, the shortage of retirements for example being most severe in fields such as physics and mathematics. "We are concerned that a

number of reports in the past have overgeneralised the problem and are pleased to note that there are some fields, such as the agricultural and earth sciences, where this is not likely to be a problem," Dr Robert M Bock, chairman of the committee which produced the report and dean of the Graduate School at the University of Wisconsin, Madison, told *Nature* last week.

However, the report says that overall, faculty retirement over the next five years will be about 5.5% of the total. A steady state model (which is expected to be reached in the mid 1990s) would imply a retirement rate of 9.2% over this period. "Thus approximately 2900 fewer positions will become available through retirements at PhD granting institutions over the next five years than would in the steady-state with the same total faculty size."

The major effect of these two trends, says the report, will be a reduction in various fields of the number of young faculty research workers. And this is potentially serious for the effectiveness of academic research, not because such research workers have a higher productivity, but because they play a vital role in introducing new ideas and perspectives into the research community.

Furthermore, the report says that "a sharp reduction in the rate of hiring of new PhD faculty might pose a serious threat to the continuation of the progress that has recently been made in expanding the participation of women and minorities in science and engineering faculties." Thus unless ways are found to ensure a continued flow of young scholars into academic positions during the transition to a steady state, "academic research in the United States is likely to suffer serious erosion in the coming decade".

Having argued the case for federal intervention, the report weighs up the arguments for the various forms that this intervention might take. Direct federal support for university faculty positions would, it says, interfere with the normal university process for selecting assistant

professors. Similarly effective early retirement and career change programmes can best be implemented and designed at the level of the individual institution.

It also argues against expanded federal postdoctoral fellowship programmes. It states that "post-docs play an enormously valuable role as producers of research, but they cannot be looked to as the principal sources of innovation and renewed vitality in academic departments". Similar reasons are given as arguments against expanding the pool of non-faculty researchers as an adequate response to what is referred to as the "vitality problem".

Setting out the various criteria required for such a response, the report says that these are best met by a programme which provides research awards in support of salary for existing faculty members for a five year period, and requires the university to use the funds thereby released to hire additional faculty.

It therefore proposes that the NSF develop a research excellence award programme, nominees for which must be full-time faculty members on regular appointments. Any nomination made for such an award would have to be accompanied by a staff development plan prepared by the department and endorsed by the university.

"This would be a considerable departure from current NSF practice under which a senior faculty member can win salary support for summer research, and sometimes for a complete academic year, but almost never for a five-year period. Nor is there currently any commitment by the university to use the funds freed in this way to employ new faculty members," says Dr Bock.

No official response has yet been given to the report by the NSF, but it is expected to announce a programme soon.

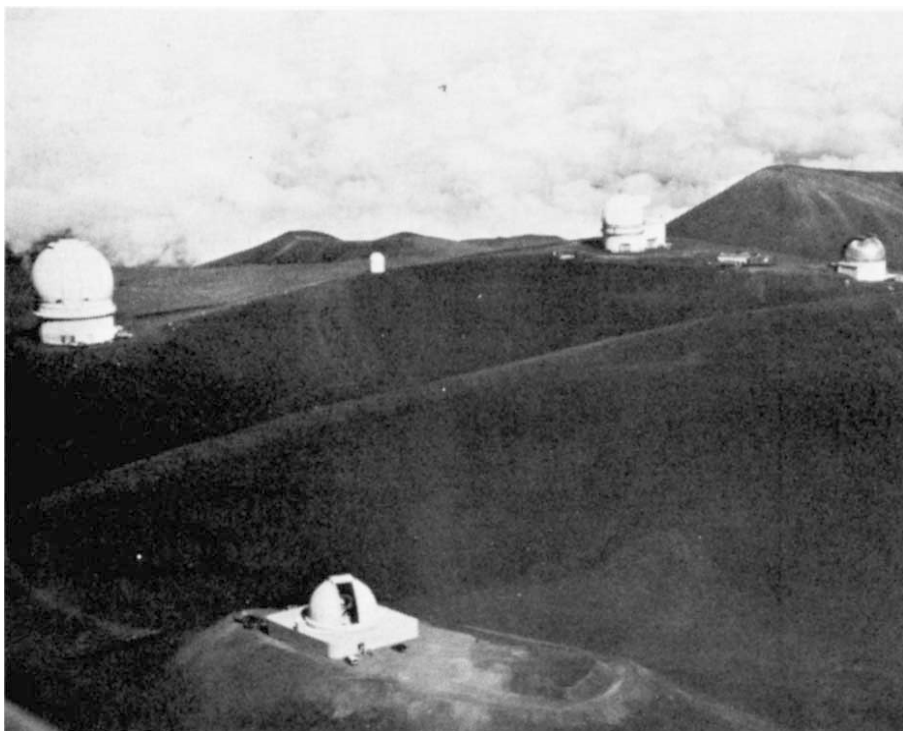
David Dickson

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Age distribution and anticipated retirements of full-time faculty at PhD granting institutions, by field of science and engineering, 1977

	Percent of Full-Time Faculty With Age			Anticipated Retirements as Proportion of Steady-State Retirements		
	> 60	56-60	51-55	1978-82	1983-87	1988-92
All fields	5.5	8.1	11.5	.60	.66	.88
Mathematics	4.5	4.2	7.6	.49	.34	.58
Physics	3.3	7.0	13.5	.36	.57	1.03
Chemistry	8.0	8.0	8.5	.87	.65	.65
Earth sciences	7.4	7.0	14.0	.80	.57	1.07
Engineering	4.0	7.8	13.2	.43	.63	1.02
Agriculture	7.5	14.2	14.0	.82	1.15	1.08
Biosciences	5.9	8.0	12.1	.64	.65	.93
Psychology	4.7	6.6	9.9	.51	.54	.76
Social sciences	6.2	8.8	11.1	.67	.72	.85
Steady state	9.2	12.3	13.0			

Source: NRC, *Survey of Doctorate Recipients*.



On top of Mauna Kea: In the foreground is the NASA infrared telescope; on the ridge (left to right) the Canada-France-Hawaii telescope, the 2.2m Hawaii telescope, and the latest arrival, UKIRT

Britain joins Hawaii summit

THE United Kingdom Infrared Telescope (UKIRT) at the summit of Mauna Kea on the island of Hawaii was opened by HRH the Duke of Gloucester on 10 October. Funded by the Science Research Council and operated by the Royal Observatory, Edinburgh, this 3.8-m diameter instrument is the largest telescope in the world designed specifically for infrared observations.

The UKIRT, however, is not the only telescope to have been built recently on Mauna Kea. The NASA 3m Infrared Telescope Facility, and the 3.6m Canada-France-Hawaii Telescope have also opened within the past three months, joining three smaller University of Hawaii telescopes already on the summit. In terms of collecting area, Mauna Kea now houses the world's largest observatory for optical and infrared astronomy.

Infrared astronomers will be looking with particular interest at the relative performance of the UKIRT and NASA telescopes, since their design philosophies are significantly different. The British instrument goes for maximum collecting area while in the NASA telescope, which cost about twice as much as the UKIRT, image quality and guiding stability are given the highest priority. The Canada-France-Hawaii telescope and the University of Hawaii 2.2-m telescope are also instrumented for infrared observations.

The UKIRT telescope cost £2.7m and was built by the Sheffield company of Dunford Hadfields, Ltd, using a Cer-Vit primary mirror which was ground and polished by Grubb Parsons, Ltd, of

Newcastle. An important feature of the design of the telescope is the thinness of its mirror, which weighs only 40% of that of a conventional telescope of the same aperture. As a result, the whole instrument was built at very substantial saving of weight and cost as compared with, for example, the similar sized Anglo-Australian Telescope. The lightweight structure has the disadvantage of lesser rigidity, but careful design and the use of computer controlled pointing has resulted in an instrument whose performance approaches that of the best optical telescopes.

The instrumentation for the telescope, built in Edinburgh, includes photometers and low resolution spectrometers covering the wavelength range 1 — 40 μm . The detectors in use are cryogenically cooled solid state devices such as indium antimonide photovoltaic detectors or gallium-doped germanium bolometers. Stray thermal infrared emission from the sky and from the telescope itself is a major problem in infrared astronomy; the optical system has therefore been designed to minimise the size and effective emissivity of the mechanical and optical components that lie in the telescope beam. In order to separate the signal due to a celestial infrared source from the background emission the secondary mirror itself oscillates at up to 40 Hz, providing the detector with near-simultaneous images of two adjacent areas of sky.

Gareth Wynn-Williams

Gareth Wynn-Williams is Associate Professor of Astronomy at the University of Hawaii

Coast guards probe mystery of missing research ship

WITHIN the next few weeks, the US coast guard hope to have developed a plausible explanation for the mysterious disappearance last December of a research vessel chartered by the University of Hawaii with seven scientists on board.

No trace of the vessel, the *Holoholo*, has been found since it set sail from Honolulu harbour on the afternoon of 9 December. The trip was one of a series planned to gather data on the oceanographic conditions off the Hawaii coast in connection with the siting of a proposed ocean thermal energy conversion (OTEC) project supported by the Department of Energy.

Evidence has been found to indicate that the vessel may have put in to shore to make bench-mark readings for some of the scientific equipment. However, the alarm was raised when it failed to make a planned rendezvous with two scientists two days after its departure from Honolulu, following a period of stormy weather.

On board the *Holoholo* at the time of its disappearance were three scientists from the University of Hawaii — including the principal investigator for the project, assistant professor of engineering Dr Gary C Niemeyer — two scientists from the University of California's Lawrence Livermore Laboratory, which has responsibility for the OTEC research programme, and two scientists from the National Oceanographic and Atmospheric Agency.

Various theories about the disappearance have been voiced during extensive hearings that have already been held in Honolulu by a board of enquiry established jointly by the coast guard and the National Transportation Safety Board. These range from the hypothesis of an explosion on board to the suggestion by some, quoting the *Glomar Explorer's* links with the CIA, that the vessel might have been captured by a foreign power.

According to a local newspaper, the Honolulu advertiser, the board that the boat sank after being swamped by heavy seas — and that the swamping may have been partly due to structural alterations made to the vessel to accommodate various species of equipment required for the research.

The safety board has already issued a set of recommendations to the University following the *Holoholo's* disappearance in which it says that some of the alterations, which included the installation of two large winches, a large spool of cable, and an A-frame cargo boom on the after part of the vessel, "may have adversely affected the

seaworthiness of the vessel”.

The board points out, for example, that although the chief scientist principal scientist was “a highly regarded investigator with considerable knowledge and experience in ocean research”, there was no evidence that he or the other scientists had expertise in vessel seaworthiness, stability, or navigation.

The board also raises questions with regard to the adequacy of procedures adopted by the university for observing guideline safety standards for research vessels laid down in May 1976 by the University National Oceanographic Laboratory System (UNOLS), based at Woods Hole in Massachusetts, an Association of Oceanographic Institutions.

It points out that although these include “an excellent array of standards and procedures which would be applicable to most research vessel operations”, and despite the fact the University of Hawaii is member of UNOLS, the guideline standards had not been applied to the *Holoholo*, which was not owned by the university but had been commissioned through a subcontract with the university’s research corporation.

As a result, says the board, the operation of the *Holoholo* apparently relied on the charter contractor for vessel seaworthiness, on the contract with the boat’s master — of whom there is no evidence or expertise in vessel stability — for safe operation, and on the judgement

of the chief scientist as to the suitability of the vessel to serve as a platform for the research projects to be conducted.

“There is no evidence that any of the guidelines standards were considered or applied by three individuals involved or by any other officials of the university or research corporation” the board says.

Officials at the university, which may be sued for substantial damages by the relatives of those on board the vessel when it disappeared, have declined so far to make any public statement about the incident, apart from saying that the specific recommendations of the safety board to prevent similar occurrences in the future” are now under study by university officials.

David Dickson

Drought could turn Dead Sea into a desert

CROSSING the Dead Sea on foot is now theoretically possible — if it weren’t for the presence of border patrols — according to Shlomo Drori, a leading Israeli expert on the use of Dead Sea resources. Following the worst drought in the history of the State of Israel, the sea has split in two at its narrowest point.

The Dead Sea’s sole feeder is the lower Jordan, which is becoming increasingly depleted by water developments, both Israeli and Jordanian. Last November, even before the onset of the drought, Yigal Allon, the former Israeli foreign minister spoke in the Knesset of the “urgent” problem of the Dead Sea. Current Jordanian water projects involve the damming of all eastern tributaries of the Jordan; when these are completed, said Mr Allon, the lower Jordan would totally disappear save as a drainage channel, the Dead Sea level would fall significantly, and the southern part of it would become a salt desert. This, as the Energy Minister Yitzak Moda’i admitted in his reply to Mr Allon, could cause “tremendous” and possibly irreversible ecological damage.

It is, however, Israel’s water schemes which are the main consumers of sources which would, without human intervention, end up in the Dead Sea. For the lower Jordan flows from the sea of Galilee, which, via the National Aquifer, serves as the water source of the whole country. According to Professor Yoram Avnimelech, a soil chemist seconded from the Technion to the Sea of Galilee Conservancy Board, considerable problems are posed to conservationists by the unique status of the Sea of Galilee, including its religious and historical associations. The normal way of treating a



Dead Sea: getting deader

lake scheduled as a drinking water source, he told *Nature*, is to fence it off, minimise development of the watershed, and try to keep the population out. This, however, is not possible in Israel, which has a limited supply of water — indeed, in the summer, every drop of water from the springs of the Upper Galilee is used three times by irrigation and fish-farming schemes before it even reaches the Sea of Galilee.

On occasion, the needs of the Conservancy seem to run counter to Israeli national sentiment. In 1968, workers at the Limnological Laboratory at Tevhag began to observe excessive levels of nitrates in the water, due not only to the use of fertilisers in the Upper Galilee but also to the uncovering of the organic soils of the Huleh valley. Since the reclamation of the Huleh marshes has attained a saga-like status in Israeli history, the obvious alternative of reflooding the Huleh was simply not acceptable, but it took several years of intensive research before a suitable strategy was devised to obviate the danger of eutrophication posed by the excess nitrates. (This is based, incidentally, on short-term sprinkling of the Huleh soil at high summer temperatures to encourage the action of denitrifying bacteria).

Professor Avnimelech, who is very much the scientific trouble-shooter for the Sea of Galilee Conservancy, also indicated another area where Jewish sentiments and

the needs of conservation may yet clash. “Planting a tree in Israel” is a tradition with considerable emotional overtones to Israeli and Diaspora Jews alike — yet recent indications, Professor Avnimelech said, suggest that over-zealous tree-planting in the Galilee watershed may be causing environmental deterioration.

Yet, however well the Water Conservancy Board husbands the Galilee resources in times of drought, the Sea of Galilee itself may be at risk. This summer, the water level fell dangerously low, and when the rain finally fell just before Jewish New Year, one official revealed that no water had been taken from the Sea of Galilee for almost four months — the country had been subsisting solely from supplies stored in auxiliary reservoirs along the aquifer.

As far as the depleted — and now divided — Dead Sea is concerned, there does exist one fairly obvious means of replacing the waters lost to human needs. This is the construction of a canal to bring in water from the Mediterranean. Israel, of course, does not have the funds to contemplate such a scheme merely as an ecological rescue operation. However, the Dead Sea lies some 400 m below the level of the Mediterranean, so that, the canal could descend via series of hydroelectrical cascades which could according to current calculations, produce up to 10% of Israel’s

present generating capacity. Although, according to Energy Minister Moda'i, electricity can still be generated more cheaply using fossil-fuelled power stations, granted the need for such a canal, power-production might make it economic.

One early exponent of such a canal was American pioneer of land conservation, William Clay Lowdermilk. In 1939, Lowdermilk formulated an "eleventh

commandment" for the future state of Israel. This reads, in part: "Thou shalt safeguard thy fields from soil erosion, thy living waters from drying up . . .". This summer's drought has made it imperative that this "commandment" should be extended to include not only the "living" waters of the Jordan and Sea of Galilee but also those of the Dead Sea.

Vera Rich

UK plans nuclear expansion

BRITAIN'S Conservative government is expected to announce details of a £10bn nuclear expansion programme within the next few weeks. The plan will involve building up to 20 nuclear reactors to supply half Britain's electricity needs by the year 2000. The Conservative cabinet is unanimous in its support for nuclear power, the only disagreements being over reactor type.

At present it is believed the American pressurised water reactor of the type involved in the Harrisburg accident is the leading candidate because it can be brought on line in five years, half the time needed by the advanced gas cooled reactor. Two public inquiries will be held before the plan can be enacted. One is a planning inquiry about the siting of the PWR, the other a full public inquiry about the necessity for a fast breeder reactor for Britain's nuclear future. Although strong opposition plan to counter it. "They will wait and see how effective the opposition is before taking counter measures" a Department of Energy spokesperson told *Nature*.

USSR questions nuclear safety: For the first time, the Communist Party has published an article on negative environmental effects of nuclear power. The article, in *Kommunist*, the party's theoretical journal, was co-authored by Nikolai Dolezhal, a prominent nuclear scientist, and Yuri Kroyakim, a relatively unknown economist. It questions the amount of valuable agricultural land that would be used, the effect on water supplies and the difficulties of storing nuclear wastes.

The article stresses that the sheer scale of the programme involving an expansion from the present level of 10 million kW to over 110 million kW by 1990 plus the construction of nuclear power stations in western Soviet cities as sources if district power heating would encroach considerably on agricultural land. Previous Soviet comment has characterised the anti-nuclear movement as being inspired by oil companies anxious to preserve their market position.

French demo blocks fuel: In a militant demonstration against nuclear fuel reprocessing, French trade unionists and environmentalists held up the unloading of

15 tons of spent nuclear fuel from Japan. Nearly 2,000 demonstrators met the ship chartered by British Nuclear Fuels Ltd when it docked at Cherbourg on 9 October with its cargo for reprocessing at nearby La Hague.

Demonstrators blocked a railway siding, severed an electric cable and put a crane out of action in a successful attempt to block the unloading. The Socialist Party and the socialist trade union federation the CFDT are demanding a national debate to prevent France from becoming "the nuclear dustbin of the world".

Waste disposal plan could take a decade: An independent waste disposal plan produced by the state of California has caught the nuclear industry by surprise. The plan calls for simulated waste to be placed in experimental vaults 1000 metre underground in variety of geological formations. Measurements would be taken on migration rates as a function of rock type, temperature, depth, and pressure and the findings compared to theoretical predictions.

The entire process would take ten years and is recognised to be the only valid way to assess present underground storage plans. The federal government accused the state government of ignoring national energy policy and of failing to keep Washington informed. The move is widely regarded as a show of strength by the anti-nuclear movement and is expected to give a boost to California governor Jerry Brown's campaign for the presidency.

Protestors march in Bonn: In the largest demonstration since the early 1930s, protestors demanded an immediate end to the development of nuclear power in West Germany. Individual columns stretched more than 2½ miles as 100,000 activists including representatives from the US, Austrian, Danish, Dutch, French and Swedish anti-nuclear movements converged on Hofgarten park in central Bonn last Sunday. Demonstrators gave a standing ovation to a speech from Kathy McCaughin of the Harrisburg Citizens Action Group who called for a stop to "the nuclear madness now". Trade union speakers were applauded when they said that more jobs would be created by alternative technology instead of nuclear power.

Joe Schwartz

Syrians blast UNCSTD over power politics

SYRIA'S delegation to UNCSTD returned home unsatisfied and a little angry. "We know that the problems of development cannot be solved with one conference, but at least we could have made a start", says Dr A W Chahid, Director of the Centre for Scientific Studies and Research in Damascus and a leading member of the Syrian delegation.

The future for the developing countries after UNCSTD, says Dr Chahid, is "just as hazy as it was before. We came back from Vienna even more convinced of the inherent biases and injustices of the existing scientific and technological order in the world".

Most of the developing countries were not prepared for the tight, hard-nosed negotiations and power play that was conducted by the developed countries at UNCSTD. Dr Chahid is particularly critical of the petty politics and the infighting of the European Community countries. "The attitude of the Nine to the proposed Development Fund was quite pathetic", he says. (A fund of \$2 billion to 1985 proposed by the Group of 77 was cut to \$250 million to 1981.) The Nine were also responsible for frustrating the good intentions of "the Nordic Group which was quite forthcoming and ready to make a reasonable contribution to the fund".

Dr Chahid thinks it was unfortunate that the Group of 77 was dominated by countries that are at the top of the development continuum. India, Brazil, Yugoslavia and other members of the 'Group of 27' of the Group of 77 who took part in the negotiations have a reasonably developed scientific and technological infra-structure. "It is these countries which stand to gain most out of the few achievements of UNCSTD".

But what about the really poor countries that suffer from the "small country problems", that do not have even the tiniest form of science and technology infrastructure? "What do they get out of UNCSTD?" asks Dr Chahid. "We must ensure that they get the lion's share of the Development Fund, little though it is".

The power and coordinating ability of the Group of 77 will be tested to the full in the setting up of the UN intergovernmental committee that will administer the Development Fund. Dr Chahid argues that if all members of the Group of 77 took an active interest in the intergovernmental committee and if there was "a very strong Group of 77 representation on the Committee", it may be possible to steer development in the Third World's favour.

However, Dr Chahid points out that many developing countries, including Syria, do not have a representative in New York.

Ziauddin Sardar

news in brief

Western nations increase proportion of R & D funds spent on military research: The proportion of research and development funds spent by the United States and Western Europe for military purposes increased from 38.5% to 39.2% between 1975 and 1977, according to a report published last week by the US Arms Control Association and the Institute for World Order. The amount of money spent on military research increased from \$12.8 billion to \$15.4 billion, says the report, which points out that the global expenditure on military purposes exceeded the rate of inflation for the seventh year, reaching a total of \$425 billion.

The report says that the R & D component of military spending is significant not only for what it has produced, but also for the continuing momentum that it gives to the arms race generally. "The steady improvement in technology has made the pace of national military spending essentially independent of the existing threat to the nation."

"In the superpower competition, each side considers it essential to develop and produce whatever is technologically possible, on the grounds that the adversary may do so. R & D is the instrument through which 'antagonistic cooperation' between the two leading adversary nations ensures a never-ending escalation of arming."

World Military and Social Expenditures 1979, by Ruth Leger Sivard. Single copies \$3.50. Available from Work Priorities, Box 1003, Leesburg Va. 22075, US.

Czechoslovakia considers ecology of uranium mining: Czechoslovakia's Minister of Energy Vlastimil Ehrenberger announced last week that the country's uranium industry had met all its increased production targets. Attention was also being given to the ecological aspects of uranium mining. In a 40-minute speech on the state of the energy industry, he told the Chamber of the People that new uranium ore deposits now ensured a "reliable base" for the country's nuclear energy industry. A large ore-processing plant, built with Soviet assistance was operating in North Bohemia. Exports of uranium to the Soviet Union were well up to target, and a scheme is being worked out for the future of the uranium industry after 1980, when the agreement signed in 1945 for supplies to the Soviet Union, runs out.

Rattled: Pennsylvania rattlesnake steaks are no longer on the menu at Dominique's restaurant in Washington DC. The rattlesnakes have been spared as the result of a letter to the restaurant's owner by C.K. Dodd, an endangered species herpetologist at the US Department of the Interior. But Mr Dodd's intercession on behalf of the Pennsylvania rattlesnakes may have cost him his job. US Secretary of the Interior, Cecil D. Andrus, a patron of the restaurant, apologised to the owner Dominique D'Ermo, for his employee's action assuring D'Ermo that the snake "is not listed as either threatened or endangered under the Endangered Species Act"; and Mr Dodd received a four page letter of dismissal.

The most serious charge against Mr Dodd seems to be that he wrote his letter on Department of the Interior Stationery. He admitted that he might have been at fault, but claimed "Even if it is against the rules most people would get a simple slap on the wrist or a reprimand". He plans to appeal. *Anne Norman*

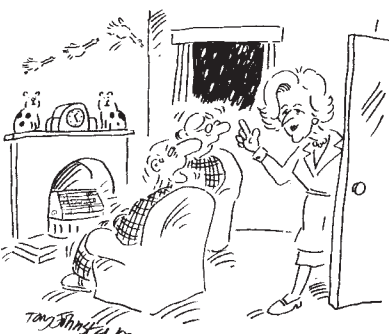
Vietnamese claimed to use poison gas: US representative Jim Leach released a report last week which claims that a number of Hmong refugees had witnessed or had been victims of seven poison gas attacks in the past year. The Hmong are a hill tribe in a remote area of Laos. They were an ally of the French and then the Americans during the wars in Indochina. According to the report, refugees from the tribe described poison gas attacks in which gas was dropped from planes in bombs or sacks which exploded 200

to 300 metres above the ground. Dozens of villagers were killed or have become ill as a result of them. The Vietnamese Embassy in London told *Nature* that the report was untrue and was an attempt to discredit the Vietnamese government "Only the US has used poison gas against the people of Indochina."

FOE takes action against "Tories cold war":

Friends of the Earth in UK are launching a major campaign to force the UK government to supply adequate heating for its citizens this winter. Calling Britain's insulation standards a "national disgrace", FOE is critical of the lack of government incentives to provide insulation for houses and of the failure of the government to live up to its election promises.

"It is ironic that in International Energy Conservation Month, the government is actually cutting its energy conservation programme while at the same time it is embarking on a potentially disastrous major expansion of nuclear power." On 18 October a FOE team will instal insulation in a London home to make it "the best insulated flat in Britain" as a demonstration project to kick off the campaign.



US firm cited for causing fertility hazards: The US Department of Labour's Occupational Safety and Health Administration last week cited the American Cyanamid Company for violating health standards by threatening the child-bearing capacity of female workers at a chemical factory in West Virginia. Last year the company admitted that it had refused to let women work in the plant's lead pigment section unless they had been sterilised, since it was concerned that the work might result in harming fetuses or affecting the women's fertility. The company says that it offered the women other jobs, although possibly at lower pay; however four women at the plant disclosed that they had been voluntarily sterilised in order to keep their jobs.

Announcing that a fine of \$10,000 was to be levied on the company for violating health and safety standards by creating such working conditions, Dr Eula Bingham, head of OSHA, said that the agency was "breaking new ground" in its actions. "What this citation is saying is that companies are required to make the workplace safe for men and women. Part of making it safe is making it safe for reproductive capacity," Dr Bingham said.

The citation requires American Cyanamid to stop insisting that female workers working with highly toxic lead be sterilised.

Socialist countries discuss joint research: Representatives of the Academies of Science of the Comecon bloc met in Tallin (Estonian SSR) last month to discuss the main directions for joint research for 1981-85. According to the protocol of the meeting, joint research is to be extended in the fields of petrochemistry, quantum chemistry, mechanics, machine-design and technological processes. A longer-term draft cooperation programme in the natural sciences up to 1990 was also discussed. The meeting formally welcomed the Socialist Republic of Vietnam (represented by its National Centre of Scientific Research and Committee of Social Sciences) into the multilateral scientific cooperation agreement of the Academies of Sciences of Socialist Countries of 15 December, 1971.

Holland's science shops for 'made-to-measure' research

Tony Ades reports on unorthodox efforts by radical Dutch scientists to match their work to the needs of society

SCIENCE shops in the Netherlands continue their steady growth and development. The annual report, to be released at the end of October, from the country's most established science shop at the University of Amsterdam, reflects an increasing scale of operations and plans for a deeper integration within the university structure.

Science shops (Wetenschapswinkels) are part of a movement that started in the early 1970s to provide a counterweight to government and industrial domination of science policy. Groups of radical scientists and students were then already organising themselves to distribute expertise and technical assistance to trade unions and other groups to whom it was not otherwise available. Mr F H P Trip, the Minister for Science Policy under the former liberal Labour government, had suggested that efforts of this sort should be supported as part of a broad attempt to democratise science.

A science shop does not undertake research itself. Its role is simply to put its clients, any group with a question, in touch with university staff able to do the research needed to answer it. The research itself is done on an unpaid and voluntary basis: faculty members must integrate it into their existing programmes, or students may undertake it as part of their dissertation. Science shops are therefore very cheap to run. Their costs need only cover advertising, copying, office expenses, and salaries for a small staff. The attraction is that the university can experiment with a novel relationship between the scientist and society, with minimal risk to the quality of education, and at negligible expense.

The movement gained impetus in early 1977 when the University of Amsterdam decided to reserve Dfl. 30,000 (£7,500) to start a science shop. The Secretary of State for Education in the present Conservative government cancelled this allocation, but the widespread protest that followed encouraged the university to take protective action: it established its science shop as an advisory committee to its governing board, a position where it remains virtually immune to political interference. The shop was duly opened in March last year, by the former minister Trip, and now has a budget of Dfl. 70,000, and a staff of four.

This year it fielded 550 enquiries, an increase of 50% on last year. Most came from unions (30%), environmentalist groups (20%) and neighbourhood organisations (15%); but a very broad range of clients was represented, including womens groups, welfare workers and

individuals. 180 of these questions have been answered in the form of full research reports or written or oral advice. A further 100 were withdrawn or unsuitable, and the remaining projects are still under way.

While it is the strongest, Amsterdam's shop was by no means the first. Science shops also operate from universities and technical colleges at Delft, Eindhoven, Groningen, Leiden, Maastricht, Nijmegen, Rotterdam, Tilburg, Utrecht and Wageningen. There is considerable variation in both the mode of affiliation to the parent university, and in the level of financial support. But support is increasing, and new shops are still being opened.

The most controversial aspect of the science shop movement is the criteria by which clients are selected. Any group or person may approach a shop, as long as they are unable to do the research themselves (or have it done elsewhere), have no financial interests in the results, and are in a position to use the results in a positive way. Because these criteria are explicitly intended to select for minority groups and exclude commercial interests, they have attracted the disapproval of the Minister of Education, Mr Arie Pais. In an open exchange with the University of Amsterdam in April this year, he agreed with the democratising role of science shops, but charged that the criteria threatened the "objective and unprejudiced" position of universities in

society. Dr Loet Leydesdorff, chairman of the Amsterdam shop, answered that science shops were in fact furthering the university's public function by re-evaluating society's need for science, and orienting research to public ends. He added that there was nothing objective or unprejudiced about the government decisions that brought so many clients to the science shop door.

The matter is expected to remain at this impasse for some time. Science shops receive warm support from unions, students and the press; and while there is certainly ambivalence within some universities, the concept is so widely regarded as rational that strong opposition is seldom voiced in public.

The research questions that clients bring in are extraordinarily diverse, both in scale and in substance. Public and industrial health are perhaps foremost, and a number of universities have set up specialised chemistry shops to analyse potentially hazardous industrial by-products and check pollutant levels. Eindhoven's physics shop has dealt with a number of clients worried about traffic noise. In one project a speed meter was built so that a neighbourhood group could obtain accurate information on how fast cars were travelling down a particular street. In such cases, which are quite common, the science shop can be very effective in redressing imbalances in the availability of science. A local authority may drag its heels over vague protests about traffic or pollution, but it will give a serious hearing to citizens groups with hard data. Without science shops such data would often remain inaccessible.

In different universities there are shops specialising in architecture, agriculture and biology. In the latter field there are numerous enquiries into the effects on



"You can't come in here": money-making industrialists are banned from science shops

animals and plants of waste dumping and weed killers, and on the environmental consequences of various farming practices.

Although science shops were originally oriented towards the exact sciences, and gained much of their early support in the health and pollution field, many of the questions now command attention from sociologists, economists and psychologists. In one recent incident, suspicions were aroused by a questionnaire that the Hilton Hotel planned to circulate among its staff. The union took advice from a sociologist and decided to boycott the questionnaire and design their own. There are numerous requests for research into the physical and mental health effects of projected highways, factory and housing developments. Second only to public health issues at Amsterdam are calls from labour unions who need to know about the likely effects of changes in management policy on conditions and employment levels in specific factories. Sometimes, prognoses on a company's balances are required.

Occasionally a science shop is called upon to provide a summary and inventory of research to date. Such requests have been received, for example, from a women's group concerned about 'the pill' and its side-effects. Often the client is simply directed to relevant literature, and the shop arranges for the material to be made available through the university library.

Some clients would exhaust the services of several think-tanks: on the books at Nijmegen are number of questions concerning energy. These include a comparative study of different energy sources, the general health risks of nuclear energy, calls for research into reactor design, how radiation norms are set, and the worldwide proliferation of nuclear facilities. Such issues should clearly be handled on a nationwide level: representatives of the different science shops meet once or twice a year, but their different internal structures and connections with their parent universities tend to inhibit coordination.

At the more light-hearted end of the scale was a question from a neighbourhood group: "Why do people like dogs? What are the psychological effects on the dog of living in a flat?" Another group, living near a milk factory, asked: "Is milk powder damaging to car-paint?" More ethereal but equally practical is the project suggested by a question put in by a political action group: "How can political action groups be most effective?"

While there is at present a mood of optimism and growth, the long term future of science shops is unclear. In Dutch universities research funds are distributed to sub-departments whose faculty plan their research programmes, with varying degrees of democracy, as a group. The group may earmark as much money as it wishes for socially-oriented research.

Dutch minister warns of research cuts



Industry in the Netherlands is slowly cutting the money it spends on research and development, and concentrating on existing work rather than new developments. This warning was given by the new minister for science policy, Dr A. van Trier (above), in announcing his science budget for 1980. The 6.1 billion guilder budget is 417 million guilders up on this year's figure, but the bulk of the increase will be borne by the government. Industry's spending will go up by only 2.5% instead of the expected 9%.

The budget makes special allowance for industrial and technological research. Energy research will give a new importance to coal, which has been largely neglected since the introduction of natural gas led to the closure of Holland's mines in the 1960s. Now, plans to import foreign coal have brought it back into favour.

Dr van Trier proposed a new "minimum" programme for nuclear research, confining it to acquiring the technology necessary for the possible building of power plants and concentrating on safety, waste disposal

and general health and environmental aspects. Only when an acceptable solution has been found for the problems of waste disposal, the safety of power plants and the risk of sabotage and terrorist action will three more nuclear power plants be built, Dr van Trier said. A decision in principle to go ahead with these was taken in 1974 but since then the argument about nuclear energy has intensified and the government now plans a broad and structured public debate, beginning next year. A decision about new power plants is not expected for two years. Research on fast breeders is to be reduced.

The science budget coincided with publication of a long-awaited energy memorandum which sets out the government's policy for the forthcoming debate. Conservation and diversification of resources are the two main features. Use of energy is expected to increase by between 52% and 86% by the year 2000 but the aim is to improve efficiency by 30% over the same period. Investment of up to 63 billion guilders will be necessary to reach this conservation goal, the memorandum says, to subsidise more and better insulation in houses and buildings and more efficient heating systems.

Alternatives like solar energy and wind power are expected to play only a minor part by the year 2000. The contribution of natural gas (now half of total use) is also likely to drop to 30% or less and will cost the consumer more. The government plans to be especially economical with the huge Groningen gasfield and switch slowly to other gas sources in the North Sea, Algeria and possibly Nigeria. The use of natural gas for electricity production will be phased out and replaced by oil and coal.

Casper Schuur

Science shops will therefore survive as long as liberal scientists dominate the faculty groups. Indeed, at Amsterdam there are plans to close the gap between scientists and the public still further. Project centres are to be set up, each one responsible for a cluster of questions. As experience with clients' needs develops, the project centres will be able to formulate research priorities and feed socially oriented science policy back to the faculty groups.

But at some universities support for the science shop concept is much more tenuous. The shops depend on a small core of enthusiastic students and staff, operating in what they regard as a hostile environment. Many of the more radical proponents of science shops feel that this lack of institutionalisation is their best guarantee of a free hand, and are suspicious that the "professionalism" at Amsterdam is a capitulation. This is

probably more a reflection of the different attitudes of the parent universities, than a real ideological split in the movement. The stress on working with rather than for the client, and the criteria by which clients are selected are strikingly consistent from one shop to another.

Yet there remain far more projects on the books than scientists willing to undertake them. It is widely agreed that the bottleneck arises from the clash between the career structure of the scientist and the interdisciplinary nature of the projects that clients bring in. The desire to do socially oriented research is quite strong: but so is the desire to have dissertations approved and papers published. The university scientist is under pressure to break new ground and to restrict research to the more tractable issues. Only the daring will tackle the open-ended questions asked on the other side of the science shop counter. □

Séveso: the crucial question of reactor safety

A technical report on what caused Italy's dioxin disaster has too many loopholes, writes **Alastair Hay**

ITALIAN chemists and engineers investigating the causes of the trichlorophenol reactor accident in which the toxic chemical 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (dioxin) was discharged over the town of Séveso on 10 July 1976 have recently completed their report. Results of the investigation, carried out on behalf of the Italian Judiciary, form the first of several reports being prepared for the prosecution case against ICMESA, Givaudan and Roche, the owners of the trichlorophenol reactor. Other reports expected soon will cover the medical, agricultural, environmental and veterinary consequences of the accident.

The dioxin discharged contaminated a large area forcing over 700 people to be evacuated from their homes. Over 100 children in the area developed mild to severe forms of the disfiguring skin disease chloracne following contact with dioxin. And over 100 Séveso women are reputed to have had abortions — either in Italy or in other European countries — fearing the teratogenic properties of the contaminant; official Italian figures only refer to 34.

Many of the medical consequences of the Séveso accident are not subject to dispute. The long term effects, however, are still a matter of controversy owing to the recently confirmed carcinogenic properties of dioxin. But the subject which still generates real heat is the design and operation of the Séveso reactor. The latest Italian report will add more fuel to the controversy. But sadly, the report has some serious faults, chiefly because it makes claims without supporting evidence and accusations which are not borne out by further investigation. If used as the basis of the prosecution case against the reactor proprietors in its present form, much of it will not stand up to a rigid cross-examination.

The process to make 2, 4, 5-trichlorophenol involves the alkaline hydrolysis of tetrachlorobenzene with sodium hydroxide at a temperature of 170–180°C, in the presence of ethylene glycol as a solvent. In the course of the reaction, and particularly at elevated temperatures, two molecules of 2, 4, 5-sodium trichlorophenolate condense to form 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin.

Dioxin formation is unavoidable during this process, but the quantity produced can be controlled by keeping down the temperature. Should the temperature rise and reach 230°C an exothermic reaction involving some of the reactor contents is initiated, heat is generated, the temperature rises rapidly and a considerable quantity of dioxin is formed. In a closed reaction vessel such as that used for trichlorophenol manufacture, the rising temperature leads to an increase in

pressure and the eventual rupture of the reactor. At Séveso, the pressure blew the safety valve, venting the reactor contents over the residential area nearby.

It is the view of the report that the reactor was unsafe and the three companies involved are charged accordingly. The companies are: ICMESA — owners of the trichlorophenol reactor; Givaudan, ICMESA's parent company; and F. Hoffmann La Roche, of which Givaudan is a subsidiary. The report's clear intention is for all three to share the blame for what happened at Séveso, and the trio have never questioned this.

Also unlikely to be challenged is the report's statement that the condition of the reactor when it was closed down at the end of the run on 10 July 1976 was unusual and that it was the first time it had been left in that condition. Givaudan say this is true. But when confronted with this in court, the company is expected to argue that the reactor should never have been left as it was, but that water should have been added to the reactor after the run to cool the contents. The company also considers that it should have been informed by its subsidiary ICMESA, of any changes in operating procedure before they were introduced.

Solvent ratios — a dangerous deviation?

One of the more serious charges which Givaudan claims it can dismiss concerns the operating conditions of the reactor. The report says that the ratio between the solvent ethylene glycol and the starting product tetrachlorobenzene was too low, and this, it insists, was a "deviation from the process originally developed in [Givaudan's] patent USP 2,509,245, where the critical nature of this ratio was expressly mentioned". In the report's judgement the alteration in this ratio increased the risk of accident.

Givaudan admits that the molar ratio of the two solvents used at Séveso was 5.6 whereas the initial patent recommended a ratio of 8. However, the patent taken out in 1950 makes clear that this ratio was important only for the yield and quality of the trichlorophenol product.

The company's claim is supported by a recent review in the Italian journal *La Chimica e l'Industria*, 61, 108 (1979). Dr Giuseppe Ferraiolo of the University of Genova refers to the critical nature of the ratio between these two compounds. Mentioning various patents for the production of trichlorophenol Ferraiolo shows that only if the ratio is less than 5 is there a danger of an uncontrolled exothermic reaction occurring. Even at a ratio of 5 the reaction remains stable up to a

temperature of 240°C.

Givaudan have confirmed they will contest one of the report's suggestions — concerning the relationship between the temperature reached by the Séveso reactor and the quantity of dioxin produced. In the conclusions of their report, the Italian chemists say that it is impossible to calculate the quantity of dioxin produced in the incident from the temperature of the reactor contents, and the concentration of dioxin in the residue left after the accident. No experimental evidence is advanced in the report to support this claim. Givaudan, on the other hand, claim that they have their own to refute it.

For the past three years the company has carried out numerous experiments in an attempt to recreate the conditions which precipitated the Séveso accident. So far this research has been unfruitful and the company is still unable to explain what happened at Séveso. However, what Givaudan does claim is that on the basis of its experiment it can predict how much dioxin is produced simply by knowing the temperature of the reactor contents. The company spokesman claimed that dioxin concentration increases with increasing temperature. This he says has already been reported (see *Nature* 273, 582; 1978). From their evidence Givaudan claim that 530 g of dioxin was produced of which 240 g was ejected from the reactor. Givaudan calculated that the dioxin content of the reactor was 115 ppm; the Italian report says the measure value is 100 ppm.

Givaudan's experiments are to continue; some results, such as those concerning the nature and properties of the chlorinated phenols produced in trichlorophenol reactors, are expected to be published shortly. Asked to put a time limit on the experimental work Givaudan's spokesman was unable to do so. In the company's eyes, he admitted, this research might be considered "unproductive work". He still regretted, however, the fact that the company had not come up with any answers yet to explain the Séveso accident.

Much of the evidence in the medical and other spheres which will be presented in court against the reactor owners will be irrefutable. This, however, is unlikely to be the case where the operation and design of the reactor is concerned. Perusal of this first report of the Italian investigators suggests that they have not done enough homework, and that their prosecution case will be anything but convincing.

It is regrettable that the chemists have not focused more attention on inadequate safety devices for example the lack of a dump tank to contain any overspill from the reactor. The chemists would have been on surer ground here, for in spite of Givaudan's claims that the Séveso accident was 'unforeseen' it happened, and the consequences were appalling. □

correspondence

Fast reactors do produce 60 times more energy than thermal reactors

SIR,—Despite Dr Jeffery's reference to "nuclear nonsense" (13 September, page 98), fast reactors do produce about 60 times more energy from the same quantity of uranium than do thermal reactors. The full reduction in uranium demand brought about by the introduction of fast reactors into a power system will not be achieved until all the thermal reactors have been replaced by fast reactors. The rate of replacement depends on many factors, including the particular rate of growth of the electricity demand, the breeding gain of the fast reactor, and the amount of plutonium needed for the initial charge of each reactor. For the particular parameters used in the calculations quoted by Dr Jeffery, uranium ore demand up to the year 2020 was reduced by a factor of 1.7, but he did not point out that some 10-15 years later there would be no need for any further uranium imports: a situation which would continue for centuries.

Dr Jeffery quotes calculations by Grainger and Merrick based on a 5.5% exponential growth rate. Such a high growth rate could not be sustained over a long period: it is about double the average growth rate over the last decade and would result by 2020 in a near 10-fold increase of the present installed generating capacity and a fuel consumption of 10^9 tonnes coal equivalent per year.

If a growth rate of much above 3% were to be maintained for one or two decades, two factors would become important. Firstly, reactor designs (which already exist) with better breeding performance than those currently specified, would be favoured. (Even the current designs have a better breeding performance than that assumed by Grainger and Merrick.) Secondly, the rate of introduction of fast reactors could be increased by fuelling some of the new reactors with U^{235} instead of plutonium. Even for a growth rate as high as 5.5% it would then still be possible to reduce uranium imports to zero within a few decades.

Yours faithfully,

B CUTTS
R D SMITH

UKAEA, Risley, UK

The National Computing Centre is not a quango

SIR,—I support the point made in your leader 'Hunting the Quangos' (2 August); Quasi Autonomous non-Governmental Organisations have undertaken many essential tasks in the public interest more effectively and for less cost than would have been the case if government departments had been used.

However, the inclusion of the National Computing Centre in your list of Quangos is incorrect. We could debate whether, or not, it ever was a Quango, depending on one's choice of definitions, but there is no doubt that today there is nothing 'Quasi' about the NCC's autonomous state.

In March 1978 an Extraordinary General Meeting changed the status of NCC from a

grant-aided body to full independence. Monies received from government sources (about a quarter of the budgeted revenue of £4.4 million) are in payment for contracts or projects undertaken on behalf of government departments. A definition of Quangos on this basis would include many independent commercial or industrial organisations which undertake government contracts.

The only formal link with government in the present NCC constitution is the Secretary of State for Industry's power to recommend two directors (out of 19) and approve changes to the constitution. In no way does this influence the independence of NCC in its day to day activities, which, incidentally, include export earnings of nearly £300,000.

Yours faithfully,

D FIRNBERG

The National Computing Centre, Manchester, UK

Farm or laboratory for male chicks?

SIR,—Lord Halsbury considers the problems of animals on farms and in laboratories to be very different (16 August, page 534). There is however, an important case where the two are directly related. I refer to the neonate male chick (*Gallus gallus domesticus*) as an alternative to mammals for large-scale toxicity tests and other laboratory purposes. Farms in the United States alone have more than 275 million laying hens of strains developed to produce eggs at a minimum cost. Since the males of these strains have no commercial value (their meat is tough and stringy) they are destroyed after hatching. Thus, the males (cockerels) are a waste product, available in numbers exceeding 200 million per year in the United States alone. The chick offers advantages of low initial cost (approximately \$0.20 each compared with \$1.50 for mice and \$4.50 for rats) and low maintenance costs. Presumably, converting cockerels from a waste farm product to a source of income could also lead to lower prices for eggs.

There is ample precedent for laboratory uses of the chick, eg, Russell *et al.* (*Toxicol. Appl. Pharmacol.* 2, 558: 1960) compared the mouse and chick in toxicity determinations and Spooner and Winters (*Int. J. Neuropharmacol.* 5; 217: 1966) reported an extensive neuropharmacological profile of the chick. It would appear to be relatively straightforward for laboratories using large numbers of mammals to compare the chick with the mammalian model under their conditions. Similarities and differences will be found; each could prove useful.

A second suggestion for reducing the consumption of laboratory mammals is employment, where feasible, of the Dixon and Massey (*Introduction to Statistical Analysis*, McGraw-Hill, New York, 1957) "up-and-down" technique. This permits a 30-40% reduction in the number of animals required for an LD50 determination and is applicable to other tests, as well. A drawback which constrain applicability is that the effect upon a given animal must be known before the next animal can be tested.

While all living things should be protected from abuse, the Cruelty to Animals Act of 1876 recognised the differential attitudes of the public toward treatment of "cuddlesome

pets" and of invertebrates. I suggest that resort to non-mammalian species, and to techniques which reduce the number of animals used in laboratories, might at least mitigate objection by the general public to the use of experimental animals.

Your faithfully,

ARTHUR CHERKIN

Sepulveda, California, US

Falsification of facts

SIR,—I have to disagree strongly with the letter by Lee Lorch (*Nature*, Vol 281, 13 September, page 98) on "Biased reporting of East European Science". My field of work is astronautics. In spite of all their activity, in this field and numerous international meetings, information from so-called Socialist bloc countries is nearly without value — ask any NASA international expert. There are some limited subject areas only (biomedical, and strictly scientific results), where my sweeping statement does not hold.

Any GDR citizen can visit the FRG, as far as the letter is concerned. It is the GDR which does not permit their citizens to travel freely. The accusation as stated by Lorch is a gross falsification of the facts as they exist now since the shameful Berlin wall was built. Since this knowledge is widely available, I hold spreading of such disinformation to be irresponsible.

"Frustration and pessimism" is an exact description of the general mood in Soviet occupied Germany. I travel there quite frequently. Possibly Lorch does not speak German or succeeded in scaring his contact persons (easily done: they know the world they are condemned to live in), so they were afraid of speaking freely.

Since I plan to continue travelling to East European countries, and to keep up the cordial personal relationship I enjoy with several scientists there, I ask that my name and affiliation be withheld.

ANON

Disillusionment with UK industry

SIR,—I wholeheartedly agree with the comments made in the article about Barry Francis and the engineering profession (9 August, page 442).

I became so disillusioned with British industry that I packed my bags and took my wife 4,000 miles west to the USA. Here I find that engineers enjoy a much better standing in society and those I encounter in industry hunger for the latest design and analysis techniques. Their appetite for using current research results contrasts so markedly with the conservative approach and outdated ideas so prevalent among British organisations. This is probably the reason why the UK is termed Britain, rather than Great Britain. We no longer have the great engineers like Brunel, Watt and Stephenson and I don't see them materialising until many of the ideas indicated towards the end of the article are implemented.

Your faithfully,

BRIAN S THOMPSON

Wayne State University, Detroit, US

news and views

Direct imaging of atoms

from J. M. Thomas

EVER since Erwin Müller first demonstrated that individual atoms could be imaged (using field-ion microscopy, (FIM)) many scientists have endeavoured to view directly the atomic structure of molecules and crystals. The reasons for wishing to do so are numerous. First, some molecules, especially those involved in biochemical processes, are reluctant to crystallise, so that it becomes necessary to evolve methods for identifying and characterising certain segments, or the entire length, of isolated molecules. Second, crystalline solids are often composed of two or more finely intergrown phases and structures; and X rays and neutrons cannot solve the structure of such solids. Third, grossly defective (for example, highly non-stoichiometric or non-randomly stacked) solids defy structural elucidation by methods based on currently available techniques of X-ray crystallography. There is, therefore, exigent need for a tool that directly reveals the local rather than the averaged structure of solids. We should know more about dislocation cores, sub-grain boundaries and other extended defects, as well as interphase interfaces, if direct imaging of atoms were possible. Moreover, this achievement would have immense repercussions in metallurgy, medical and surface science as well as in solid-state physics and technology. Progress towards some hitherto inaccessible goals was reviewed at a recent symposium.*

Scanning transmission electron microscopy (STEM) is, in many respects, a natural complement to, and a direct descendent of FIM. Scattering of heavy atoms on light (carbon or boron) supports shows up readily when a minute pencil (<5 Å diameter) of electrons is scanned across a thin specimen; and there is no doubt from the work of A.V. Crewe (Enrico Fermi Institute, Chicago) and others that atomic resolution of isolated heavy atoms is

readily possible. Unfortunately for biologically significant molecules the electron dose required to observe single heavy atoms (such as iodine, mercury or platinum which may be used to tag certain specific parts of a macromolecule) is at least an order of magnitude more than the dose which damages the macromolecule itself. Extensive beam damage to unstained molecules such as myokinase, valinomycin and vasopressin in STEM (F.P. Ottensmeyer, Ontario Cancer Institute) prompted A. Klug (MRC Laboratory of Molecular Biology, Cambridge) to ask whether the ashes of 'cremated' molecules are reminiscent of the original. As this question is still unresolved Klug argued cogently that biologists' time could be better spent trying to induce hitherto non-crystallisable macromolecules to form ordered two or three-dimensional aggregates so that they could be investigated by other methods.

For crystalline and defective solids STEM has two serious inadequacies: it is not easy, as pointed out by J.M. Cowley (Arizona State University), to orient specimens precisely, nor, at least on commercially available instruments, to record the diffraction pattern. These are essential prerequisites for high resolution structural imaging. It was, therefore, not surprising that most of the symposium was devoted to high resolution electron microscopy (HREM) by conventional (that is transmission) instruments.

V.E. Cosslett (University of Cambridge) described the performance of the high voltage (600 kV) high resolution microscope built as a collaborative venture between the Cavendish Laboratory and the Cambridge University Department of Engineering (W.C. Nixon). The minimum resolvable distance in a conventional electron microscope is proportional to $C_s^{1/4} \lambda^{1/4}$ so that improvements arise when the coefficient of spherical aberration (C_s) of the objective lens and the electron wave length, λ , are decreased (that is, accelerating voltage is increased). The strenuous attempts made to construct high-stability microscopes operating at 200, 600, 1,000 and 3,000 kV — all at present

functioning in a few centres throughout the world — are therefore understandable. But how trustworthy is the projected image (assuming that the specimen suffers no appreciable beam damage or gives rise to minimal inelastic scattering)? When specimens are ultrathin, so that the weak phase object approximation holds true, interpretation is relatively straightforward, provided due regard is paid to the contrast transfer function of the objective lens. With thicker specimens multiple electron scattering is extensive and the observed image may bear no resemblance to the projected structural features of the object. Fortunately, a powerful procedure devised by Moodie (CSIRO, Melbourne) and Cowley and known as the 'multislice' method, enables the electron microscopic image — and its dependence on specimen thickness and the extent of lens defocus — to be calculated with considerable confidence. This method constitutes a means of matching computed with observed images. If the agreement is satisfactory, credence is given to the assumed structure that was fed into the calculation. The overall procedure of structure determination by electron microscopy is, therefore, akin to the trial and error method extensively adopted by X-ray crystallographers before the advent of direct methods. This restrictive state of affairs does not engender complacency, and is one of the reasons why theorists such as Moodie have developed a way, which he outlined, of achieving, under specific experimental conditions, direct inversion from the back focal plane.

L.A. Bursill (University of Melbourne) described how, by judicious use of 100-kV microscopes, structural resolution of less than 3.0 Å can be achieved. He demonstrated how novel features in certain naturally-occurring tunnel structures and incipient ferroelectrics are revealed by microscopic investigations. He also examined the question of 'model sensitiveness' of the image match in relation to the structure of the rather

*Nobel Symposium 47 on 'Direct Imaging of Atoms in Crystals and Molecules', was organised by P. Kierkegaard, A. Magneli, L. Kihlberg and M. Lundberg, and held at the IBM Nordic Education Centre, Lidingö, Sweden in August, 1979. The proceedings are to appear in a forthcoming issue of *Chemica Scripta* and also in a special publication edited by L. Kihlberg.

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enigmatic platelets known to occur (on {100} planes) in diamonds.

The trial and error method for determining crystal structures by HREM was strikingly illustrated by N. Uyeda (Institute for Chemical Research, Kyoto University) for a new phase of V_2O_5 ; by M. Sundberg (Arrhenius Laboratory, Stockholm) for a new tetragonal tungsten bronze consisting of pentagonal columns in a composition $W_{24}O_{68}$; by L. Kihlberg (Arrhenius Laboratory) for a fascinating range of intergrowth tungsten bronzes (M_xWO_3 with $M = K, Rb, Cs$ and so on, and $x \leq 0.10$) consisting of hexagonal tunnels; and by S. Horiuchi (National Institute for Research in Inorganic Materials, Japan) on a new derivative of silicon nitride ($Si_3N_4 \cdot Y_2O_3$). None of these structures could have been solved by X-ray methods.

The image matching method was shown by J.M. Thomas (University of Cambridge) to be capable of distinguishing which of two possible new chain structures in silicate minerals exists as an extended, two- or three-dimensional defect inside nephrite jade, work which illustrates the power of the multislice method. A. Bourret (Centre d'études Nucléaires de Grenoble) likewise showed how structural details of one-dimensional faults in elemental semiconductors (Si and Ge) could be clarified by image matching; and S. Ijima (Arizona State University) has put the image matching approach to good use in his studies of the layer by layer build-up of graphitic carbon sheets. Even when no multislice calculations are carried out, structural insight may sometimes be gained by careful comparison of observed and 'expected' images, a fact vindicated by the work of O. Krivanek (University of California, Berkeley) and J.L. Hutchison (University of Oxford). The former, working with the Kyoto 500-kV microscope, showed that, as had been surmised, sub-grain boundaries in the Ge are made up of 5- and 7-fold rings: the latter has succeeded in clarifying the structure of both polytypic variants in certain perovskites and of Aurivillius phases. Horiuchi presented convincing proof that 1,000-kV high resolution images of $Nb_{12}O_{29}$ (a block structure) surpassed those taken with the best commercially available 100-kV microscopes. He also demonstrated that, by imaging ferroelectric crystals of Bi, Ti, NbO_{21} along two or more high symmetry directions, the point group and hence the real space structure, may be determined directly. Resolution is sufficient to indicate, on the microscopic image, that the heavy atoms in the Aurivillius phase $Bi_2W_2O_9$ are not arranged on undistorted cubic lattice.

Two other studies illustrated the importance of high voltage HREM. N. Uyeda (Kyoto University) showed superb micrographs of hexadecachlorocopperphthalocyanine, which by adroit sample preparation (Uyeda *et al.* *J. appl. Phys.* 43, 5181; 1972) will form pillars of

overlapping molecules running essentially in the direction of projection. Apart from the central Cu and the peripheral Cl atoms, the outlines of the porphyrin rings, in this exceptionally beam-stable organometallic material, may be satisfactorily imaged. Thomas, with the aid of micrographs taken on the Cambridge 500-kV instrument (Jefferson *et al.* *Nature* 281, 51; 1979) demonstrated how a point-to-point resolution of about 2.7 Å may now be comfortably achieved, thus making it possible to identify corner-sharing SiO_4 tetrahedra, and hence the backbone repeat patterns of the pyroxenoid minerals wollastonite and rhodonite.

It is most unlikely that solid state reactions, other than the kind described by R.J.D. Tilley (University of Bradford) who discussed the way in which electron irradiation generates so-called crystallographic shear (CS) planes in WO_3 when the latter, through oxygen loss, becomes progressively non-stoichiometric, will prove generally amenable to direct, *in situ*, investigation by HREM. In the words of J.S. Anderson (University College of Wales, Aberystwyth) the absence of 'eyewitness' procedures compels us to rely on the coroner's report in reconstructing the scene of the crime. With the aid of micrographs taken by his collaborator Sian Crawford Davies he gave a fascinating account of the quasi-homogeneous oxidation reactions that take place within solids derived from the archetypal block structure Nb_2O_5 , allegedly "the most flexible material in the whole of inorganic chemistry". Electron diffraction patterns of reactant and products in these systems are sometimes indistinguishable; but high resolution images enable one to chart (post event) the series of subtle, unit cooperative jumps (of the kind strikingly illustrated in other systems in a time-lapse movie film shown by H. Hashimoto, Osaka University) that may occur along a favoured line of atoms within these remarkably compliant materials. HREM studies clearly permit a 'projected' radial distribution pattern surrounding the locus of chemical reaction to be constructed in real space, from 'samples' consisting merely of a few tens of atoms. Post event rationalisation by J.V. Sanders (CSIRO, Melbourne) guided by HREM studies of hydrodesulphurisation catalyst particles has brought to light several unexpected facts, including the occurrence of encapsulating layers (a few atoms thick) of MoS_2 . Comparable approaches have been adopted by Krivanek on silicon-silica interface. A. Howie (Cavendish Laboratory, Cambridge) dealt with amorphous materials, the radial distribution functions of which (extracted from X-ray data) do not adequately elucidate the local structure. Howie pointed out that HREM also runs into serious difficulty here, largely because of the problems stemming from the inherent unreliability of the projection procedure.

Nevertheless, valuable progress has been made and Howie concentrated on how various experimental and processing (phase randomisation) approaches have been of value in the study of 'amorphous' metals, silicon and carbon.

Intermetallic systems were discussed from two viewpoints. S. Amelinckx (SCK/CEN, Mol), in an extensive series of unusual examples (dealing with alloys such as Au_4Mn , Au_5Mn , $Au_{11}Mn_4$) demonstrated how HREM, supplemented by selected area diffraction has greatly enlarged our knowledge of imperfectly ordered alloys. He emphasised how, under certain circumstances, there can be advantages in utilising superlattice spots to form relatively low power images rather than to push resolution to its limit (by taking the 'fundamental' diffraction spots). L. Stenberg's discussion (based on his work with S. Anderson) of some tetrahedrally close-packed alloys (such as Mo_3CoSi , Zr_4Al_3 and $MgCu_2$) strongly suggested that, as a result of advances in HREM, the structural inorganic chemist and the metallurgist will soon be brought closer. The concepts of CS, anti-phase boundaries and chemical twinning, which have proved valuable in interrelating structures among binary and ternary oxides seem to be equally useful in discussing these close-packed alloys. □

From ligands to legumes

from A. W. B. Johnston

THE interaction of atmospheric nitrogen with synthetic catalysts, and the interaction of *Rhizobium* with clover are two distantly related areas of research united by the phenomenon of biological nitrogen fixation. These two topics have been studied in depth by two eminent workers, Professor J. Chatt (Director of the Unit of Nitrogen Fixation, Sussex) and Dr P. S. Nutman (Head of the Soil Microbiology Department, Rothamsted) respectively, and to mark their impending retirements the Phytochemical Society of Europe organised a symposium* which included contributions on the whole spectrum of current nitrogen fixation research.

At the heart of the nitrogen fixation process lies the enzyme nitrogenase. W. H. Orme-Johnson (University of Wisconsin, Madison) considered the arrangement of the two metals, Fe and Mo, which are present in the protein subunit that is responsible for the binding and reduction of nitrogen. The Mo is present in a metal cluster that also contains Fe (MoFe

*An International Symposium on Nitrogen Fixation was held at the University of Sussex on 17-19 September, 1979, sponsored by the Phytochemical Society of Europe.

cofactor); the majority of the Fe is present in Fe—S clusters that are in a highly reduced state and which are in a less exposed position than is the MoFe cofactor.

A point made by both J. Chatt and R. H. Burris (University of Wisconsin, Madison) was that nitrogenase has different affinities for the substrates N_2 , H_2 and C_2H_2 depending on its oxidation state. Since the reduction of C_2H_2 to C_2H_4 is often used to assay nitrogenase activity it follows that it is not straightforward to extrapolate the values obtained from this assay to calculate absolute amounts of nitrogen that are being fixed.

Burris also referred to the fact that in *Rhodospirillum* and in *Azospirillum* the success in isolating the Fe-containing nitrogenase component is variable in different laboratories. It seems that there is an inactive form of the protein which can be activated by enzymatic removal of moieties (possibly adenine) and that the occurrence of the active or inactive forms depends upon the growth medium.

Nitrogenase needs a source of energy and electrons for reducing power. H. Haaker (Agricultural University, Wageningen) proposed that in *Azotobacter vinelandii* and in the bacteroids of *Rhizobium leguminosarum* the transport of electrons to nitrogenase depends upon the electrical component ($\Delta\psi$) of the proton motive force since there was a correlation between the value of $\Delta\psi$ and nitrogenase activity. In free-living nitrogen fixing bacteria, NH_4^+ is a potent repressor of nitrogenase but in the case of *Rhizobium* bacteroids, nitrogenase is relatively immune to NH_4^+ repression. Interestingly, the addition of NH_4^+ to *A. vinelandii* caused a drop in $\Delta\psi$ but the $\Delta\psi$ of *Rhizobium* bacteroids was immune to the addition of NH_4^+ .

There are some bacteria that are doubly blessed in that they can fix nitrogen and can obtain the energy for fixation by photosynthesis. J. R. Gallon (University College of Swansea) considered whether in the case of blue green algae, photosynthesis could directly provide ATP and reductant to nitrogenase. Some evidence to support this came from the finding that inhibition of photosystem II rapidly inhibited nitrogenase activity in *Gloeocapsa*. However nitrogenase activity was quickly restored and it was suggested that this recovery was due to the switch on by another, non-photosynthetic, source of energy.

Bacteria that are fixing nitrogen liberate gaseous H_2 due to the reduction of protons by nitrogenase. Many species possess a hydrogenase that can be used to recoup some of the energy that would otherwise be lost. H. J. Evans (Oregon State University) discussed the hydrogenase system in *Rhizobium japonicum* in which the enzyme is sufficiently active to allow autotrophic growth on hydrogen as sole energy source. The fact that when hydrogenase-deficient

Squirrels kill kids but are nice to relatives

from John Krebs

BELDING'S Ground Squirrel (*Spermophilus beldingi*) conceals a vicious habit beneath its charming, furry coat. P. W. Sherman has studied a population of 250-300 of these little beasts high up in the Sierra Mountains of California since 1974, and he reported on some of his results at the XVI Ethological Congress.* The squirrels hibernate underground beneath the snow for about 8 months of the year and have a brief reproductive season during the months of May-August. Their life is a tough one as might be expected from such an extreme environment: 54-93% of juveniles and 23-68% of adults die during winter hibernation, and the survivors have to face the dangers of numerous nocturnal (coyotes, and bears) and diurnal (raptorial birds, weasels and coyotes) predators, as well as the risk of being run over by a passing car. These kinds of natural hazards are well known as causes of death in natural populations of many species, but more unusual is the fact that a considerable number of young squirrels die as a result of infanticide. About 8% of all young are dragged from their burrows by adult squirrels and killed before their mothers can come to the rescue. Sherman's detailed analyses of the causes and consequences of infanticide lead him to the following conclusions.

Adult females and yearling males are the commonest killers. Adult females kill when their own young have been destroyed by predators: the bereaved mother moves to a new place (choosing carefully to go to a place less susceptible to predator attack) and tries to kill the young of a mother in the area. If she is successful in killing young the female settles near the burrow of her victims. It seems, therefore, that infanticide by adult females is a form of competition: the female removes potential future competitors by disposing of her new neighbour's young. The young male killers, unlike the adult females, eat their victims, and Sherman suggests that the benefit derived is simply that baby ground squirrel is a good source of protein for a growing lad. A young male's future survival and reproductive success in this highly polygynous species depends heavily on fighting ability and hence body size (Sherman in *Natural selection and social behaviour: recent research and new* John Krebs is a Lecturer in Zoology in the Edward Grey Institute of Field Ornithology, University of Oxford.

*The XVI International Ethological Congress was held in Vancouver, BC, Canada, 18-27 August, 1979.

theory. (Alexander & Tinkle eds.) Chiron Press 1979).

Thus infanticide is far from being a pathological behaviour shown by a few squirrels under abnormal conditions; it is a normal part of the day-to-day business of competition and survival. Female squirrels with young are highly territorial during the period in which their infants are helpless to defend themselves, and mothers with large territories have the lowest risk of infanticide, which suggests that territory defence might be an anti-infanticide device.

Belding's Ground Squirrels are not, however, universally nasty: close relatives are nice to each other. The squirrel society is matrilineal. Females pass on bits of their defended home area to daughters while males disperse well away from home soon after weaning. Sherman's accurate data on kinship enabled him to show that close female relatives cooperate with each other in a number of ways. They share parts of their territories, do not attack each other very often, and help each other in guarding their burrows against infanticidal intruders. Mothers also put themselves at a demonstrable risk when a predator approaches by giving alarm calls to warn close relatives of the danger (Sherman *Science* 197, 1246; 1977). Squirrels without close relatives in the vicinity (males and transient females) do not give alarm calls, which is consistent with the fact that they do not stand to make a genetic profit by self-sacrifice.

Sherman's unique and impressive data show that altruism (or nepotism as he prefers to call it) occurs between close relatives. This is to be expected since an altruist's genes are propagated not just through children but also through other relatives, and some would argue that Sherman's data are a good test of the theory of kin selection. In a sense, however, the theory is not testable. It is a logical generalisation of the theory of Natural Selection and is not one of a number of alternative, refutable hypotheses. However, an example of the kind of interesting question which arises from kin selection theory is Sherman's observation (*Nat. Hist.* 88, 80; 1979) that while mothers, daughters and sisters show nepotism towards one another, more distant relatives such as aunts and nieces or grandmothers and granddaughters do not. As Sherman puts it, the reasons for this limit to nepotism remain an enigma.

mutants were used to inoculate soybeans the yields were about 30% less than when wild-type strains were used shows that the hydrogenase system is of real agronomic importance.

The developments in the genetics of nitrogen fixation were discussed by a number of speakers. The most detailed analyses have been on the *nif* gene cluster of *Klebsiella pneumoniae* and these were

considered by M. J. Merrick (Unit of Nitrogen Fixation, Sussex). Fourteen genes have been identified and these are organised into eight transcriptional units. A useful tool in the study of gene control in this system involves the insertion into the different *nif* operons of a derivative of bacteriophage Mu carrying the structural genes but not the promoter of the *Escherichia coli lac* operon. Thus expression of any *nif* transcriptional unit can be determined by assaying β -galactosidase production. By such means it was shown that when *K. pneumoniae* was derepressed for *nif* expression different *nif* genes were turned on at different times. The allocation of particular proteins (seen on two-dimensional gels) to particular *nif* genes was demonstrated in posters by J. Houmard (Institut Pasteur) and by W. Klipp (University of Erlangen). Klipp also showed that the DNA corresponding to the *K. pneumoniae nif* region was significantly more dense than the typical value for the chromosome of this species and is also flanked by two sets of inverted repeats. It was suggested that the *nif* genes may have been acquired relatively recently by recombination with some other bacteria.

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The close relationship of *nif* DNA from different nitrogen fixing species in widely separated taxonomic groups was in fact demonstrated by F. Ausubel (Harvard University). A segment of the *nif* operon containing *K. pneumoniae* structural genes for nitrogenase has been cloned and been found to hybridise with DNA from 8 different nitrogen fixing species but not to 12 other, non-fixing species. In the case of *R. meliloti*, hybridisation was specifically with plasmid DNA, a conclusion also supported by data presented by A. Kondorosi (Hungarian Academy of Sciences, Szeged). J. E. Beringer (John Innes Institute, Norwich) reported that in *R. leguminosarum*, some of the genes that are required for nodulation and for fixation within the nodules have been located on the same resident plasmid found in a strain of this species.

The past 10 years have seen a great rise in interest in biological nitrogen fixation, stemming largely from the awareness that the production of commercial nitrogenous fertiliser makes significant demands on fossil fuel energy. This interest has been reflected in the real developments that have been made in many areas of research in this field and these were amply demonstrated in many of the contributions that were given at the symposium. □

protein) contains hydrophobic peptide sequences in the B region (40K), located at the C-terminal of the whole toxin molecule (P. Falmagne, Université d'Etat à Mons, Belgium). Presumably diphtheria toxin first binds to surface receptors (as yet unidentified) and then inserts into the phospholipid bilayer by more stable transverse associations mediated through the hydrophobic peptide sequences (see Boquet *et al. Proc. natn Acad. Sci. U.S.A.* **73**, 4449; 1974). Pappenheimer believes that such an association requires the cleavage of a C-terminal sequence of the B fragment which possibly is facilitated by the unusually high incidence of basic residues in this region. Additional features of diphtheria toxin action on sensitive cells comes from study of mutant proteins (Pappenheimer). Toxin (CRM 45) of abnormally low molecular weight (45K) isolated from a chain-terminating mutant contains an enzymically active A fragment (22K) but lacks much of the B region sequence required for interaction with surface receptors. CRM 45 toxin contains the hydrophobic sequence, however, and is about 1-2% as toxic as normal toxin when injected into chickens and is equally toxic when injected into the brain of mice where the cells chiefly affected are Schwann cells. Fragment A itself, lacking the hydrophobic sequence of CRM 45, is at least 100-fold less toxic in this system emphasising the importance of the hydrophobic sequence in mediating toxicity. Pappenheimer suggests that the intact diphtheria toxin, the CRM 45 protein and fragment A are taken up into cells by different mechanisms of steeply decreasing efficiency. Evidently even the latter inefficient uptake is sufficient to kill a cell because it had been calculated that one or two toxin molecules in the cytoplasm are sufficient.

This high sensitivity probably also explains the recent success in synthesis of hybrid toxic molecules. Despite one negative result of Chang *et al. (J. biol. Chem.* **252**, 1515; 1977) who found no killing of mammary cells by a diphtheria A subunit — human placental lactogen complex, more recent reports have shown that diphtheria A subunit coupled with concanavalin A (Gilliland *et al. Proc. natn Acad. Sci. U.S.A.* **75**, 5319; 1978), *Wisteria floribunda* lectin (Uchida *et al. J. biol. Chem.* **253**, 6307; 1978), and antilymphocyte globulin (Thorpe *et al. Nature* **271**, 752; 1978) or ricin A subunit coupled with human gonadotropin (Oltmann & Heath *J. biol. Chem.* **254**, 1022; 1979) or concanavalin A (Yamaguchi *et al. J. natn Cancer Inst.* **63**, 1387; 1979) are cytotoxic, although at high doses and over rather long periods. Presumably, the processing of the conjugates is mediated after binding of the surface binding ligands and leads to penetration of the toxic A subunits. It is not clear whether this uptake

How do toxins penetrate cells?

from R. C. Hughes

THE death of the Bulgarian broadcaster, Georgi Markov, apparently by a ricin bullet, received considerable press coverage recently. In less sombre vein the cytopathological effects of ricin and other toxins are of wide interest since they hold out some hope for site-directed killing of tumours and may also throw some light on the mechanism of action and entry into cells of molecules such as hormones and growth factors.

At a recent workshop meeting*, F. Stirpe (Istituto di Patologia Generale, Bologna, Italy) described the effects of injection of ricin into rats as severe necrosis of sinusoidal (Kupffer) cells of the liver and of the red pulp of the spleen. Abrin and modeccin are related toxins, binding to similar glycoprotein receptors as ricin. While modeccin affects the liver, abrin specifically brings about severe necrosis of acinar pancreatic cells. Like many toxins, ricin, abrin and modeccin bind to cell surface receptors, enter the cell and exert a cytoplasmic effect, in these particular cases by inhibition of protein synthesis. All three toxins apparently block protein synthesis

at an identical site on 60 S ribosomes and are structurally very similar, consisting of two polypeptide subunits. One subunit (B chain) binds to cell surface galactose residues following which the toxic A subunit is taken up into the cell and transported to the ribosomes (see Olsnes in *Transport of Molecules in Cellular Systems* (ed. Silverstein) Dahlem Conference, Berlin, 103; 1978). Since the A subunits of ricin, abrin and modeccin are biologically indistinguishable the interesting target specificity described by Stirpe must be due either to small differences in the preferred surface receptors for each toxin expressed on different cell types or to differences in the steps subsequent to binding. Stirpe suggested that these steps may include processing or degradation of the toxins, and such processing may be essential in mediating cytotoxicity or if carried to excess, may destroy biological activity.

The mechanisms by which bifunctional molecules such as ricin traverse the cell surface membrane and transport the toxin portion of the molecule to the cytoplasmic site of action are poorly understood. A. M. Pappenheimer (Harvard University) presented compelling evidence for the direct transfer of the toxic principle of diphtheria toxin. Diphtheria toxin (a 62K

*The Workshop 'Toxins with intracellular targets' was organised by S. Olsnes (Norsk Hydro's Institute for Cancer Research, Oslo) and P. Boquet (Pasteur Institute, Paris) as part of the 6th International Symposium on Animal, Plant and Microbial Toxins held on 19-24 August, in Uppsala, Sweden.

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is similar to that described for the intact diphtheria toxin or is more closely analogous to the less efficient mechanisms by which CRM 45 or diphtheria fragment A penetrate the cell.

The uptake of ricin into cells may proceed in a way analogous to the Pappenheimer model for diphtheria toxin. Ricin undergoes a conformational change after binding to oligosaccharide receptors that may facilitate direct transfer, for example by exposure of hydrophobic segments. S. Olsnes (Norsk-Hydro's Institute for Cancer Research, Oslo) and R. C. Hughes (National Institute for Medical Research, Mill Hill) showed that ricin action is inhibited at low temperatures. Mosquito cells and baby hamster kidney cells are protected significantly from ricin toxicity at 28°C although binding to cells and inhibition of protein synthesis in cell free systems are as effective at 28 as at 37°C (T. Butters & Hughes). Since mosquito cells grow optimally at 28°C the temperature effect is likely to be attributed to the ricin molecule itself, possibly in the conformational change mentioned above. One cold sensitive structure could be eliminated as contributing to the temperature effect. Colchicine, which disrupts cytoplasmic microtubules in hamster fibroblasts, has no significant 28°C effect on ricin toxicity. Cytochalasin B does protect cells after prolonged treatment before the challenge with ricin suggesting a possible involvement of microfilaments in ricin penetration into cells.

Bundles of microfilaments directly under the surface membrane are believed to have a role in receptor-mediated pinocytosis by contraction of the plasma membrane containing the surface absorbed ligand. However, the role of endocytosis in mediating entry of toxins is still controversial. Receptor-mediated endocytosis of toxins has been observed directly in several systems but such experiments use very high levels of toxins for visualisation, usually much higher than the cytotoxic dose. It is then not clear whether the toxin molecules seen enclosed in endocytotic vesicles within the cytoplasm are those responsible for the toxic effect by leakage of toxin from just one vesicle. Endocytosis is clearly involved in uptake of lipoproteins into cells at the specialised surface sites, enriched in the specific receptors, known as 'coated pits' (Goldstein *et al.* *Nature* **279**, 679; 1979). However, lipoprotein uptake is geared to the lysosomal degradation of proteins and release of cholesterol and it would be premature to assume similar mechanisms operating in toxin action.

Nonetheless, J. L. Middlebrook (US Army Medical Research Institute of Infectious Diseases, Fort Detrick) showed that both diphtheria toxin and *Pseudomonas* exotoxin A may enter some cells by receptor-mediated endocytosis. Local anaesthetics and lysosomotropic agents such as

chloroquine protect cells from toxicity. Similarly, G. T. Keusch (Tufts University, Boston) showed that lysosomal stabilisers or drugs affecting microfilament or microtubule function inhibited the effect of *Shigella* exotoxin on sensitive cells while DEAE-dextran, a stimulator of endocytosis, significantly increased the cytotoxic response of the cells to the toxin. Keusch proposed that a limited degradation of *Shigella* exotoxin in the lysosomes is a necessary step in cytotoxic action, which is reminiscent of the proposals for ricin by Stirpe and for diphtheria toxin by Pappenheimer. Such processing seems to be a common feature of many models of toxin action. Clearly, lysosomes are specialised for such protein degradation but specific limited proteolysis at cell surfaces is still an attractive alternative.

The evidence at present suggests strongly that toxin action mimics the normal mechanisms of action of hormones and growth factors. In some cases similar or identical surface receptors are involved and it is quite likely that the subsequent steps in mediating these diverse biological effects may turn out to be similar. □

Meteoritics — life begins at seventy

from A.E. Fallick and C.T. Pillinger

It is now known that nearly 20% of the naturally occurring elements have isotopic variations which are not the result of nuclear reactions within the Solar System, and therefore it was something of a novelty for the recent meeting of the Meteoritical Society in Heidelberg* to close without a report of a new isotope anomaly. However, interest in the subject is not declining. In fact the opposite is true, and accordingly two reviews were devoted to this exciting area, G.W. Lugmair (San Diego) dealing with the state of the art and W. Hillebrandt (Munich) considering the challenge presented to theories of nucleosynthesis.

The group led by Peter Eberhardt (Physikalisches Institut, Berne) has perhaps best demonstrated the painstaking approach now needed to track down the location of anomalies. Since 1974 various members of this department have been investigating the occurrence of a component of the rare gas neon (called neon-E) in a number of meteorites. After an extremely detailed study starting from 6 g of the Orgueil carbonaceous chondrite, M.H.A. Jungck and Eberhardt have isolated a few hundred micrograms of an unidentified, but probably carbonaceous, component which on stepwise heating releases neon having an isotopic

composition $^{20}\text{Ne}/^{22}\text{Ne} = 0.008$ and $^{21}\text{Ne}/^{22}\text{Ne} = 0.0006$, which means that it is practically pure ^{22}Ne . One possibility is that neon-E is the daughter product of ^{22}Na , an isotope with a 2.6 year half-life.

Much interest is still focused on the Allende meteorite. For example, E.K. Jessberger and E. Dominik (*Nature* **277**, 554; 1979) recently reported a potassium-argon age of 5.08×10^9 year for an Allende inclusion. Isotopic measurements on potassium by W. Stegmann and F. Begemann (Mainz) have shown a composition within $\frac{1}{2}\%$ of the terrestrial value, which discounts the possibility that the extraordinarily high age is an artefact explicable in terms of an enhancement in ^{40}K .

Isotopic anomalies are certainly no longer confined to meteorites. Various workers, including J.F. Kerridge (Los Angeles) and R.N. Clayton (Chicago), have for some time been advocating a secular change in the nitrogen isotopic composition of the solar wind. M.H. Thieme and Clayton now report a new low δ value of -110% (relative to atmospheric gas) for nitrogen in an Apollo 17 lunar soil. Whilst astrophysicists are presumably aghast at having to explain nitrogen isotopic fluctuations of 30% in the source region of the Sun, Clayton admits that the onus is on the experimenter to verify conclusively the data.

Also on the Moon, an examination of the siderophile trace element pattern of Apollo 17 black glass spheres by J.W. Morgan (Reston) suggests that these glasses are not purely of volcanic origin, as has been proposed. The relative abundance of seven out of eight elements points to the possibility that a particular type of iron meteorite splashing into a lava lake could have been partly responsible. Speaking from the floor, Ed Anders (Chicago) recalled that when such an origin was proposed a number of years ago there was a degree of incredulity amongst the audience, but he felt the evidence now presented had reopened the debate.

Inevitably a number of 'old faithfuls' reared their heads. Although first recognised in 1802 and named in 1863, chondrules remain an enigma. Klaus Keil (Albuquerque) eloquently dismissed as many origins for these as there are students of the subject minus one — and in as many seconds — during his review of the field. On the basis of textural and chemical evidence, and laser-induced melting simulations, he favours a secondary impact origin rather than some primary process. New food for thought for chondrule students, however, came from Kurt Fredriksson (Smithsonian Institute) who, because of improved techniques in manufacturing ultrathin polished sections, was able to observe a carbonate inclusion in the centre of a chondrule from the Allegan meteorite.

In the new areas of endeavour it was good to see a number of papers devoted to

*The 42nd annual meeting of the Meteoritical Society was held at Heidelberg on 3-7 September.

recently discovered Antarctic meteorites. One interesting point made by L. Schultz (Mainz-Paris consortium) was that a sample collected in the Allan Hills must have had a preatmospheric radius of only 1-2 cm. It was previously thought that such small samples did not survive passage to Earth. At the other extreme several investigators, including members of a Chinese delegation, described work on the Kirin meteorite, a four-tonne chondrite which fell recently in the People's Republic. A survey of the ancient literature of China by Tu Guangzhi (Institute of Geochemistry, Guiyang) reveals many credible records of phenomena attributable to falling meteorites, the earliest being in 2133 BC. We hope that China's future participation in meteoritics will be as prolific as its past.

Another hope for the future is the planned chemical investigation of cometary dust particles during possible fly-by of Halley's

comet and rendezvous with Temple 2. B.-K. Dalmann (Max Planck Institut für Kernphysik, Heidelberg) described various experiments for the secondary ion mass spectrometer on the rendezvous mission, including an attempt to measure stable carbon isotope ratios. Another possible source of cometary debris for analysis, suggested by Dave Brownlee (University of Washington, Seattle), is the collection of micron-sized particles from the stratosphere by U2 aircraft. Perhaps study of these grains could reveal the carbon isotopic ratio of comets if a sufficiently sensitive technique were available.

Finally as an encouragement to those of us who have yet to make a significant contribution to science, the Meteoritical Society chose to honour Paul Ramdohr (Max-Planck-Institut für Kernphysik, Heidelberg) with its Leonard Medal. Although Professor Ramdohr claims an interest in meteoritics from his first months as an undergraduate in 1909, he did not commence the work for which the medal was awarded until after his 'retirement' in 1960 at the age of seventy! □

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Is there anyone out there?

from J. Tarter and B. Zuckerman

"WHERE are they?" This question attributed to Enrico Fermi held centre stage during a full day symposium on 'Strategies for the Search For Life in the Universe' at the XVII General Assembly of the International Astronomical Union.

The first topic under discussion was ' N ' the number of communicative civilisations that currently exist in our Milky Way Galaxy. I. Shklovsky (Sternberg Astronomical Institute) suggested that the choice of a value for N may be a very large number ($\geq 10^9$). Considerably less optimistic was M. Hart of Trinity University who argued that N is likely to be very small (~ 1). Hart pointed out that a calculation of N by means of the so-called Drake equation is essentially impossible owing to the orders of magnitude uncertainty in many of the terms. A better way to estimate N , according to Hart, is to assume some value for N and enquire about its effect on observables. In particular, he asked, if N is very large then, "where are they?". That is, if there are a million civilisations in the Milky Way then at least one of them would have colonised our Solar System by now since the time to colonise the entire Galaxy, with nuclear rockets for example, is far less than the age of the Galaxy. Kuiper referred to Fermi's question as the "big gun in a small arsenal" of arguments in support of $N \sim 1$.

F. Drake of Cornell University occupied the middle ground. The negative results of

searches for radio beacons, and radio astronomy in general, imply that N is not very large. But he also argued against $N \sim 1$ and the interstellar colonisation idea on the grounds that colonisation is not cost effective. A better way to improve the quality of life per dollar spent is to remake your own planet and/or solar system. This idea was countered by Kuiper who argued that colonisation would increase the raw material base and by S. von Hoerner of the US National Radio Astronomy Observatory who remarked that luxury makes life nice but "luxury is never cost effective".

P. Morrison of MIT summarised the discussion of N , noting that little new has appeared on the scene since he and Cocconi published the first SETI paper in *Nature* 20

years ago. We have only four 'facts' to go on: Hart's 'fact A' (they are not now here on the Earth), the negative results of radio searches, the presence of life on the Earth, and its absence on Mars. We are still not in quantitative control of the problem, Morrison observed, since calculations of N give 2σ limits between 10^2 and 10^9 . As he did so long ago, Morrison argued for modest experiments with existing radio telescopes to bound the problem observationally.

The second session was devoted to radio searches for extraterrestrial intelligence (SETI). V. Troitsky of the Scientific Radiophysical Institute in Gorky outlined Soviet plans for a fully automated and continuous SETI programme using widely separated antennas on the ground linked with a 10-m dish in space. Troitsky and Shklovsky were two members of a sizeable Soviet delegation at the General Assembly which convened in August in Montreal, Canada. B. Zuckerman (University of Maryland) described recent and ongoing SETI programmes in the USA and Canada and S. Gulkis (Jet Propulsion Laboratory) summarised a SETI programme proposed for inclusion in the NASA FY'82 budget. This 6-year programme, a joint endeavour between NASA's Ames Research Center and JPL, would attempt to optimise the probability of detection using existing radio telescopes in conjunction with new low noise receivers and extremely large digital spectrum analysers.

Jesse Greenstein chaired a discussion on the prospects for detection of simple forms of life elsewhere in the Solar System and the discovery of extrasolar planets. He seemed to respond to Morrison's lament over the lack of progress, noting that SETI as a science was in the awkward position of being "respectable but not yet funded". At least in the case of planetary detection, it was clear that funding would produce definitive answers to a limited set of interesting questions in the near future. G. Soffen from NASA-HQ reviewed the biological ramifications of "life as we know it" and the possibility of recognising "life as we don't know it". T. Owen from the State University of New York pointed

100 years ago

Change of Colour in Frogs

It is certainly a common opinion in this part of the country that when frogs become of a bright yellow colour fine weather may be expected. The brightness of colour can scarcely be due to the presence of sunlight, for frogs of a bright yellow may frequently be found in cellars, wells, and other dark places. Throughout the past summer and up to the present time, I have noticed that the frogs in this neighbourhood have been of an extraordinarily brilliant yellow tint. Again and again have I heard the country people, working in the hay or corn fields, under the unbroken canopy of cloud, remark — "We must be going to have fine weather now, for

look at the colour of the frogs." These forecasts proved the reverse of successful.

At the commencement of "the rains" (say, beginning of June), in the island of Bombay, after the first showers, when a little water lodges in the depressions of the old-quarry tanks, the frogs issue from the crevices of the trap-rock to spawn, when the *males* (some of which are 18 inches in length from tip of toe to end of digit) assume a bright mustard-yellow colour, while the females remain brown as usual; and this change of colour takes place so rapidly, and the frogs are so numerous, that, with the falling of the showers, the bottom of the quarry becomes suddenly yellow.

From *Nature* 20, Oct 16, 580; 1879.

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out the spectroscopic potential for detecting abundant quantities of O_2 or other disequilibrium atmospheric constituents, indicative of the presence of some form of life.

As for finding extrasolar planets, indirect schemes based on state-of-the-art astrometry, interferometry, radial velocity measurements and the wide field CCD cameras on the Space Telescope were described in turn by G. Gatewood (Allegheny Observatory), D. Currie (University of Maryland), I. McLean (University of Arizona) and W. Baum (Lowell Observatory). Comparing these techniques D. Black of Ames noted their complementarity due to the intrinsically different dependences on stellar mass and planetary size and orbit sampled by the various methods. He concluded that the milli-arcsecond spatial resolution and the 10 m s^{-1} velocity precision needed to detect a Jupiter-Sun system in the local solar neighbourhood are at hand. However direct detection schemes in the visible or infrared and the micro-arcsecond/ 10 cm s^{-1} precisions required to detect an Earth-Sun system must await the next generation of instrumentation. While this entire field looks extremely hopeful it was suggested by the participants that it may require a 'dialectic unification' to get two different groups of astrometrists to agree on the question of the existence of a planet orbiting around Barnard's star. □

The nucleolus at Weimar

from E. G. Jordan

THE workshop* opened with an outline of our present understanding of nucleolar structure given by M. Bouteille (University of Paris) in which he emphasised the importance of the identification of the discrete pale-staining islands of the nucleolus as the nucleolus-organising regions. These areas which have been given many different names in the past, are clearly distinguishable from the two major parts of nucleoli, the fibrillar and the granular components. P. Hernandez-Verdun (University of Paris) showed that in micronuclei induced with colchicine treatment no organisation of nucleolar material into nucleoli occurred in the absence of such a pale-staining 'fibrillar centre', hereby shown to be functionally related to the nucleolus organisers. C. Mirre (Faculty of Medicine, Marseille) reported that the number of fibrillar centres contributing to the generation of a new nucleolus in meiotic cells was the same as the number of organising chromosomes

The answer lies in the blood

by Mary Lindley

Tiny, dried up bloodstains of indeterminate age may not be the biochemical geneticist's dream, but for those who work in the forensic world they are routine specimens. The continual challenge of obtaining more information from less and less material was in the minds of delegates at a recent international gathering of forensic haemogeneticists*. With the elucidation of the kinetics of the reaction between antigen and antibody, it has become possible to identify blood group antigens immunologically with greatly improved efficiency. P.J. Lincoln (London Hospital Medical College) explained that by careful manipulation of pH, ionic strength and the amount of antiserum used, it is possible to identify an antigen on a single bloodstained thread, so that even a minute bloodstain can provide sufficient material to examine for several antigens. Sixteen red cell antigens, including those of the A,B,O and Rhesus systems, can be identified by an elution technique. The thread is incubated with a specific antibody, which is subsequently eluted and examined by a sensitive agglutination test. Antibodies corresponding to the antigens in the bloodstain bind to the dried blood, are recovered by elution and detected in the eluate. The serum markers of the Gm and Km system, determining differences in immunoglobulin constant regions, can be detected by inhibition tests. Antiserum is incubated with the bloodstained thread and if antigen is present, the antibodies are absorbed and the activity of the antiserum inhibited.

Some antigens remain detectable by these methods for only a short time, while others such as ABO can be detected after many months, or even years. The age as well as the amount of material available thus determines the antigens used to establish the origin of a bloodstain. A combination of a few of these antigens, Lincoln said, can distinguish between the bloods of two individuals in nearly every case.

Inhibition tests also form the basis for detecting HLA antigens in bloodstained thread, described by D. Hodge (London Hospital Medical College). This work is very preliminary and so far only the HLA-

A antigen has been tried, largely because it is well represented in European and Caucasian populations and because good antisera were available. Best results have been obtained when several antisera have been used in parallel to type a stain. Even assuming success, Lincoln said, HLA typing of bloodstains is unlikely to become routine in casework in the near future but it could provide a valuable specialised procedure.

More than 20 systems of enzyme polymorphisms can be identified in bloodstains as a result of recent improvements in electrophoresis and the introduction of isoelectric focusing, described by B.J. Culliford (Metropolitan Police Forensic Science Laboratory, London). Using 18 such systems, he and his colleagues know that when they identify a bloodstain according to its profile of enzyme polymorphisms it has come from no more than one in 1,800 people. When a specimen is very meagre, it can be tested sequentially for two or more systems. G.B. Divall (Metropolitan Police Forensic Science Laboratory) said that one piece of bloodstained thread can be used first to identify Gm, Km and enzyme polymorphisms by electrophoresis, and then A,B, and O by an absorption-elution technique.

Early results reported by B. Brinkmann (Institut für Rechtsmedizin, Universität Hamburg) suggest that measurement of enzyme activities could be a guide to the age of a bloodstain. In test bloodstains the activities of acid phosphatase, phosphoglucomutase, adenylate kinase, adenosine deaminase, and phosphogluconate dehydrogenase declined, rapidly at first and then more slowly, at rates correlated with the enzyme phenotype and the time and temperature of previous storage. Much more has to be learnt about the effects of environmental conditions, however, before any routine application for assessing the age of bloodstains would be possible.

J.L. Thomsen (University Institute of Forensic Medicine, Copenhagen) reported his success in identifying the Y chromosome of leukocytes extracted from bloodstains. He has managed to obtain sufficient leukocytes to treat them with the fluorescent stain that reveals the Y, the only chromosome identifiable in dead cells. In spite of the difficulty of the procedure, Thomsen suggested that it could offer a reliable means of sex determination in blood. His claim to have obtained no false positives was greeted with some scepticism however.

*The Eighth International Congress of the Society for Forensic Haemogenetics was held in London on 23-27 September, 1979. The congress presidents were Professors B.R. Dodd and C.P. Engelfreit. The proceedings will be published by the Society and further information can be obtained from Dr P.J. Lincoln, Department of Forensic Medicine, London Hospital Medical College, Turner Street, London E1.

Mary Lindley is an Assistant Editor of Nature.

*The Sixth European Nucleolar Workshop was held in Weimar on 1-6 July, 1979 under the auspices of the European Cell Biology Organisation (ECBO) and was organised by Dr. S. Rosenthal of the Central Institute of Molecular Biology, Academy of Sciences GDR, Berlin.

involved. Many workers agreed that the use of the silver staining procedure identifies these 'fibrillar centres' and hence the nucleolus organisers. However, the

specificity of the silver stain is known to depend critically on the conditions used and some workers reported silver staining of structures other than nucleolus

articles

A deep-sea hydrothermal site on a strike-slip fault

Peter Lonsdale

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Submersible exploration of a young scarp in the San Clemente fault zone, at 1,800 m in the California Borderland, discovered tall piles of hydrothermal barite and dense colonies of large benthic animals. Phenomena along this active strike-slip fault zone resemble those at hot springs along the axes of mid-ocean ridges.

DIVE 355 of the US Navy submersible DSV-4 Seacraft, a half-sister of DSRV Alvin¹, landed at 1,805 m on the sandy suprafan of the Navy deep-sea fan², 70 km south-west of San Diego, California, in the South San Clemente Basin. The original intent of the dive was the geological examination of large-scale scour marks that have been cut by turbidity currents and mapped by a deep tow instrument³. Navigation was by echo-ranging to the Loran C-positioned mother ship, and by piloting on the deep tow chart. After travelling east across the hummocky fan surface for 1 km, the submersible obliquely ascended the 50–75 m scarp of a south-east trending fault (Fig. 1). The scarp has a clearly defined foot lapped by fan turbidites, and an abrupt crest at the top of a 60° slope. Although acoustic profiles (Fig. 2) indicate that the scarp truncates back-tilted beds of layered sediment, no semi-lithified outcrops were visible; the scarp surface is covered by silty mud, with signs of local mass-movement of unconsolidated sediment down its steepest segments. Both on the fan surface and on this part of the scarp the type and abundance of animal life seemed normal for basins in the California Borderland, with mobile holothurians, ophiuroids and quill worms the most obvious megafauna.

After climbing to the crest of the narrow fault ridge, Seacraft turned south and recrossed the scarp. At a position of 32°13.6' N, 117°43.5' W, about 500 m south-west of the first crossing, the foot of the scarp is marked by an uneven line of cones, columns and irregular piles of mineral deposits (Fig. 3a, b). They generally rise 2–3 m above the sediment surface, but one large mass has an estimated height of 10 m. Some of the deposits are white, and have a very uneven surface with many fingers and protuberances, but many of the larger masses have a smoother dark brown surface and a patchy dusting of sediment. The dark crust was easily broken by the submersible's manipulators, to reveal friable white interiors. Higher up the scarp, extending about half-way to the crest, are smaller white deposits, commonly seen as irregular fingers less than 1 m long projecting through the mud (Fig. 3c, d). Around some of these deposits the buff-coloured sediment bears thin patches of dark-brown soot-like material, the water within a few centimetres of white surfaces has a milky appearance, and there are dense colonies of large tube worms and other animals.

The mineral masses proved to be made of barite crystals. Although we lack direct evidence of anomalous bottom water, having had no water sensors or samplers on Seacraft, the phenomena at this site are interpreted as effects of mineralised thermal springs discharging along the fault line.

After almost an hour of photography (with hand-held 35 mm cameras through the observer's port) and sampling, the dive was curtailed because of mechanical difficulties. How far the baritic sinter and tube-worm clusters extend along the fault line was not determined, but it is at least 100 m.

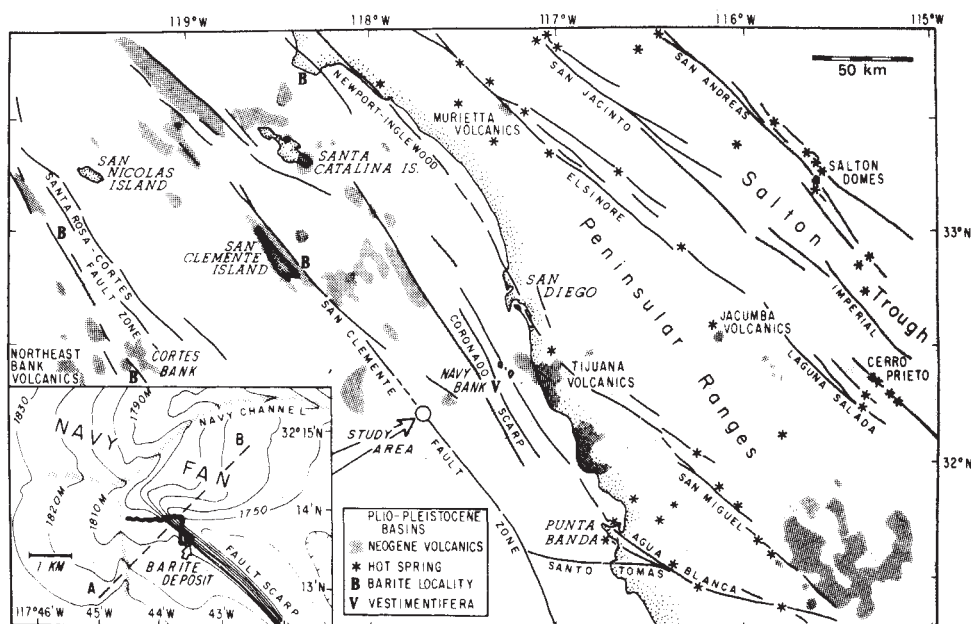
Regional setting

The scarp at Navy Fan has been created by a component of dip-slip motion along the predominantly strike-slip San Clemente fault zone. Local vertical displacements are common along California strike-slip faults, with the uplift alternating from one side to the other along the fault trace; 100 km north-west of our study area tilted San Clemente Island has been formed by relative uplift of the opposite side of the fault. The fault ridge we examined preceded and steered the post-22,000 yr BP growth of Navy Fan², but continued motion on the fault line may be indicated by low scarps and lineations mapped by side-scan sonar on the fan surface north-west of the ridge³. We lack seismic profiles across the dive site, but the basement comes within 100 m of the sea floor at a crossing of the fault 5 km to the south-east⁴. The nearest sampled basement outcrop is at Navy Bank, where andesites have been dredged⁵; the basaltic andesites of San Clemente Island have been dated at 15–16 × 10⁶ yr BP⁶.

The San Clemente fault zone is but one of the several simultaneously active shear zones that make up southern California's broad strike-slip 'boundary' between the Pacific and American plates. It was once thought to connect with the Agua Blanca fault zone³, but recent seismic reflection surveys⁷ indicate that the principal fault trace is well offshore at Punta Banda, and continues south-south-west along the margin of Baja California (Fig. 1). The Miocene lavas of San Clemente Island are manifestations of widespread basaltic volcanism that was associated with plate-boundary tectonism in mid-Miocene to Pliocene times. The tholeiites, alkali basalts and basaltic andesites throughout this province are notable for their unusually high contents of barium^{8,9}: for example, tholeiitic and transitional basalts from the Jacumba volcanics have up to 0.3% barium, more than 200 times the average for mid-ocean ridge tholeiites⁸.

Quaternary igneous activity has probably been restricted to the Salton Trough, where incipient seafloor spreading may be taking place¹⁰, but the geothermal gradient remains above average in much of the region¹¹. There are many subaerial thermal springs with temperatures of 30–60 °C, concentrated

Fig. 1 Location map, showing principal Quaternary faults (after refs 5, 7, 33 and 34), offshore barite occurrences and the Vestimentifera type-locality¹⁸. Inset, with bathymetry from a deep tow survey³ shows submersible track (solid line) and the position (A-B) of the 3.5-kHz profile of Fig. 2.



content. Despite its thinness, this crust helps hold the loose mass of barite crystals together, and must increase the resistance and persistence of the tall barite cones.

Benthic fauna

The most prominent animal life on the barite deposits and the intervening sediment slopes are dense tangles of tube worms. Many of the colonies near the large, brown-crust deposits at the foot of the scarp may be dead: their crooked tubes are dusted with sediment and thickly festooned with epizoa, and no bodies were seen protruding from the tubes (though they could have been retracted inside). Near the fresher white deposits higher on the slope (for example, Fig. 3b, c) the tubes (many of them more than 1 m long and averaging 1 cm in diameter) tend to be straighter and less encumbered with epizoa, and several had dark red 'plumes' protruding from their tips. The animals were sampled, but their soft parts were not preserved; from their tubes and growth habit they are identified as the vestimentiferan *Lamellibrachia barhami*. Descriptions of this gutless annelid (?)^{18,19} rely on specimens observed and collected by submersible at 1,125 m on the foot of Coronado Scarp, a fault scarp on the east side of San Diego Trough about 40 km from the Navy Fan site. Its only known close relatives are the Vestimentifera clustered around some thermal springs on mid-ocean ridges²⁰.

Unidentified bluish-grey organisms form apparently gelatinous masses draped over some barite surfaces and many of the chitinous tubes of the Vestimentiferae we photographed. Some of the sampled tubes had Bryozoa colonies. Large stalkless crinoids were attached to the tops of several barite cones (see Fig. 3b). Carpeting the sediment surface near some irregular white projections of barite were clusters of gastropods 4–5 cm long (Fig. 3c) and the valves of bivalve molluscs up to 10 cm long (Fig. 3d). Other animals seen around the barite deposits, but with an abundance not noticeably greater than elsewhere on the fan, include ophiuroids, holothurians, several types of fish and a large blue octopus (nestling in a barite concretion).

Interpretation and implications

The sinter-like deposits of barite on the floor of a Borderland basin are precipitates from mineralised springs along an offshore part of California's strike-slip fault system. Their evident capacity for carrying large amounts of barium in solution indicates that the springs were discharges of reduced hy-

drothermal fluid. Recent precipitation is attested by the fragility of the deposits and the absence on many surfaces of any coating of manganese oxide or sediment. The best signs of continuing active hydrothermal discharge, in the absence of direct temperature measurements, are the abundance of animals whose feeding habits attract them to thermal springs, and the milky appearance of bottom water in contact with some barite piles, reminiscent of the hydrothermal plumes at mid-ocean spreading centres²⁰. By extrapolation, other places in the Borderland with nodular or massive barite are inferred to be sites of past or present thermal springs, as is the type-locality for *Lamellibrachia barhami* on the Coronado Scarp.

Several features of the continental margin site explored by Seaciff are comparable to the more spectacular deposits of oxides, silicates and sulphides and the dense clusters of large organisms found around thermal springs at axial fissures and strike-slip faults on mid-ocean ridges^{20–24}. Seawater-based hydrothermal fluids that have reacted with young oceanic tholeiites at the Galapagos spreading centre²⁰ have the high barium enrichments predicted from laboratory studies²⁵, but no local accumulations of barite have been described. Barite precipitation has been reported from an actively spreading marginal basin²⁶, while dispersed barite crystals of probable hydrothermal origin are found over a large area of the East Pacific Rise²⁷, and in the Red Sea's metalliferous muds²⁸, where calcium sulphate is much more abundant.

The environment of thermal springs along the San Clemente fault one is different from those at accreting plate boundaries in that the geothermal gradient is much lower and the deep convective circulation must be much weaker. However, an active hydrothermal system has already been inferred for the San Clemente fault zone²⁹ from the pattern of conductive heat flow, which includes measured fluxes as high as $3.2 \mu\text{cal cm}^{-2}$. The rocks and sediment available for reacting with hot, reduced seawater are also different from the sediment-free oceanic tholeiite of most mid-ocean ridges. Dominance of barite precipitation is attributable to the high concentration of barium in the local Neogene volcanic rocks.

In the convective circulation proposed for the San Clemente fault zone, seawater that penetrates shattered bedrock would have its sulphate reduced by reaction with volcanic rock, from which barium would be stripped by the chloride solution. A

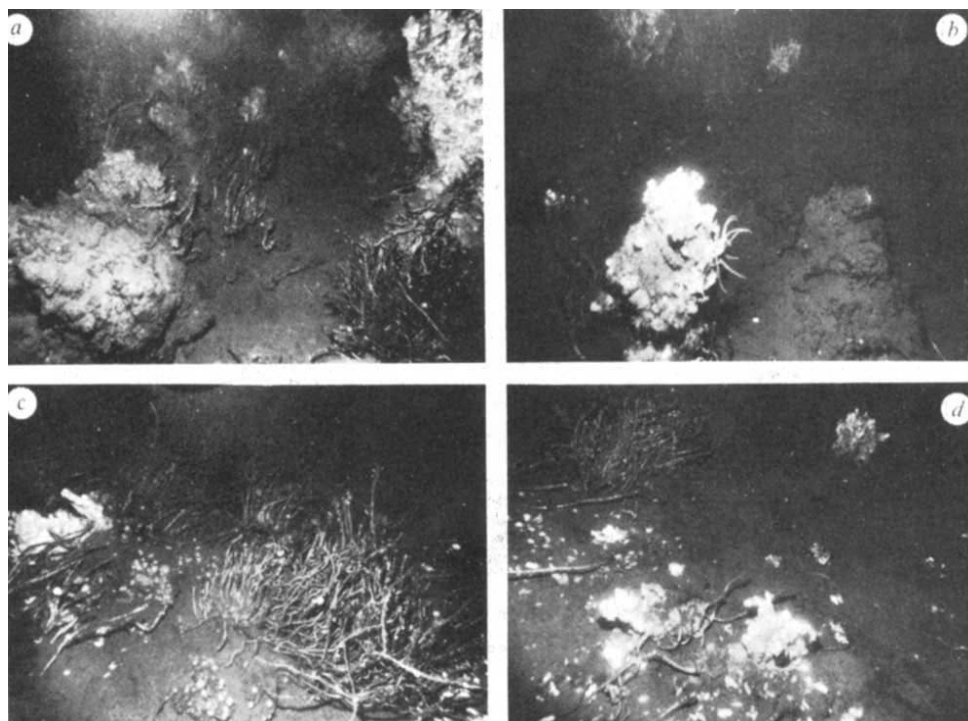
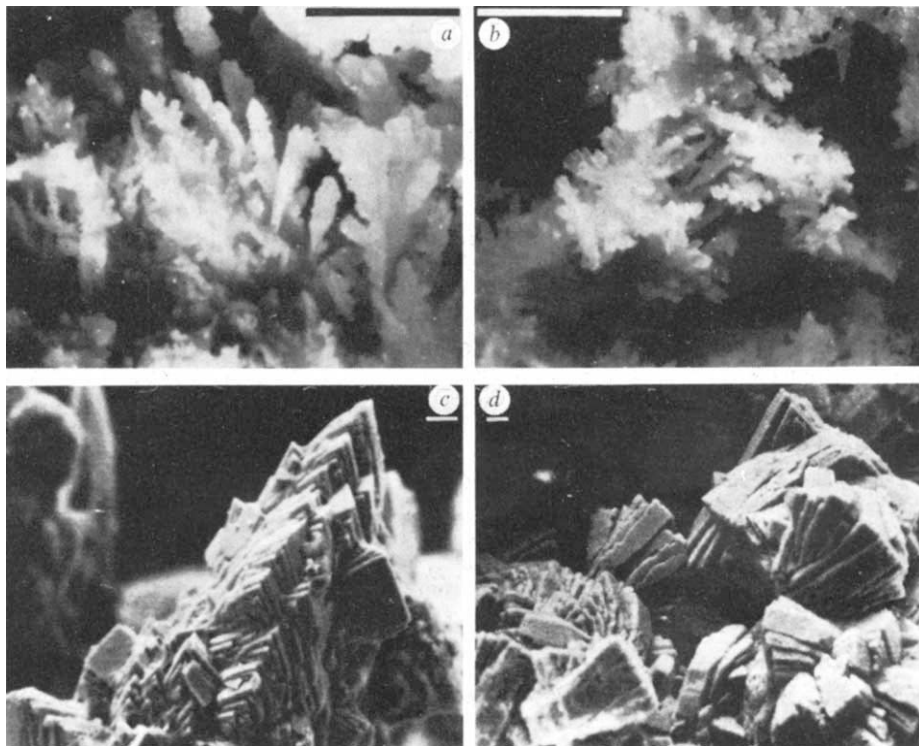


Fig. 3 Reproductions of colour photographs of the barite deposit, taken with a hand-held 35 mm camera through Seaciff's observation port. Field of view is about 3 m wide in mid-field. *a*, Irregular barite mounds 1–2 m high at the foot of the fault scarp, with tangled tubes of probably dead Vestimentiferae. *b*, Contrasting appearance of two barite mounds, one white and irregular (with a large stalkless crinoid), and one darker, smoother and veneered with sediment. *c*, the 30° lower slope of the fault scarp, with protruding 50 cm high 'fingers' of white barite, abundant tube worms (some definitely alive, with protruding red bodies), and clusters of white gastropods (on the sediment, barite, and some Vestimentifera tubes). *d*, Irregular clumps of barite, surrounded by milky water (poorly seen in this black and white reproduction), clusters of empty clam shells, and living Vestimentiferae. Irregular patches of dark, sooty sediment visible on the slope.

Fig. 4 Internal structure of barite crystal masses, as seen by a reflected light microscope (*a, b*; scale bar, 1 mm) and scanning electron microscope (*c, d*; scale bar 10 μ m); platy crystals form spires and frondose structures projecting into internal cavities.



conduit for discharge through the relatively impermeable sediments is provided by the fault, which probably resembles strike-slip faults on land in being a belt of shearing 100–200 m wide, overlying a similar width of crushed bedrock. Precipitation of barite, which is undersaturated in normal seawater, would occur where barium-rich hydrothermal fluids mix with sulphate-rich bottom water. The large number of separate mounds of barite indicates a more diffuse discharge than occurs at ridge-crest hot springs, where there is no sediment cover to fractured bedrock. Because the crystal masses are so porous the precipitated mounds do not impede the discharge and seal the system. Those mounds with fresh white exteriors are believed to be slowly leaking barium-rich water, and growing barite crystals on their surfaces; their dynamics may be similar to the slow discharge through the porous deposits of manganese, iron and silica at the Galapagos Mounds Hydrothermal Field^{20,30}. Manganese oxide precipitation on the mounds apparently occurs after outflow of barite-precipitating fluid has ceased, and does effectively decrease surface permeability and seal the discharge.

Iron and aluminum in the barite deposits has probably also been leached from underlying igneous rocks¹⁵, which are also the most likely source of encrusting manganese. The dark sooty sediment nearby may be a loose precipitate of manganese oxide. Elsewhere, baritic sinters are often associated with sulphide ores of more valuable metals. In northeastern Honshu, barite oc-

as onshore hot spring deposits, offshore nodules, and as the capping of Kuroko ore bodies—stratiform deposits of copper-lead-zinc sulphides formed at bathyal depths by Miocene hydrothermal precipitation in offshore basins overlying rhyolite and andesite^{31,32}. Just as hydrothermal vents on the East Pacific Rise may now be producing mineral deposits similar to the Troodos-type massive sulphides²², so thermal springs in the basins of the California Borderland may be modern ore-depositing counterparts of the Kuroko hot springs. The subaerial hot springs of Baja California have previously been suggested as the loci for active accumulation, at depth, of sulphide ores³³; the prospective value of seawater-based hydrothermal systems like those in the Borderland is greater because of their enhanced ability to transport metals in chloride complexes. Establishing the abundance of mineralised thermal springs in the Borderland will require high-resolution observations along the fault zones there; in the submarine environment they are unlikely to be as far apart as the 10–50 km that separates fault-zone thermal springs on the adjacent semi-arid land (Fig. 1).

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Modification of the oxygen-isotope record in deep-sea cores by Pleistocene dissolution cycles

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The glacial-interglacial amplitude (GIA) of oxygen-isotope curves in sediment cores from the Atlantic Ocean is much higher than the amplitude in the Pacific. This difference is explained by nonsynchronous CaCO_3 dissolution cycles during the Upper Pleistocene that amplify the GIA in the Atlantic and reduce the GIA in the Pacific. Efforts to assess palaeotemperatures in the Pleistocene using the stable isotope technique must consider the effects of dissolution.

MUCH of our knowledge on the Pleistocene epoch and its glaciations comes from oxygen isotope studies of foraminiferal skeletons from deep-sea cores. In addition to being a powerful stratigraphic tool¹⁻⁴, the oxygen isotope record provides information on the timing of glacials, sea-level fluctuations, palaeotemperatures, palaeosalinities and ice accumulation and melting during glacials and interglacials¹⁻⁹. Deep-sea cores also display cyclic variation in CaCO_3 content. These CaCO_3 cycles have a periodicity similar to that of the Upper Pleistocene glacial-interglacial fluctuations and are probably caused by CaCO_3 dissolution¹⁰⁻¹³. The purpose of this article is to show how CaCO_3 dissolution cycles have modified the oxygen isotope record in deep-sea cores.

CaCO_3 dissolution cycles during the Pleistocene

The mechanism that causes CaCO_3 cycles in deep-sea cores is the subject of much debate and one reason for this is the lack of synchronicity between the Atlantic and the Pacific with respect to the timing of these cycles. In the Atlantic CaCO_3 content is high during interglacials and low during glacials, while in the Pacific the opposite is observed¹⁰⁻¹⁶. Variation in the CaCO_3 content of oceanic sediments can be caused by changes in CaCO_3 productivity, CaCO_3 dissolution and dilution by non-carbonate material. All these mechanisms and some of their combinations have been suggested to explain the CaCO_3 cycles and to account for the difference in timing between the Atlantic and the Pacific¹⁰⁻¹⁶.

Broecker¹⁴ and Berger¹¹ have convincingly shown that in the Pacific CaCO_3 cycles are caused mainly by dissolution. Their conclusions are based on CaCO_3 mass balance and on micro-palaeontological criteria. Luz and Shackleton¹² provided additional evidence for CaCO_3 cycles in the Pacific being caused by dissolution, and also showed that these cycles lag a few thousand years behind the onset of glacials and interglacials. However, several authors suggested that CaCO_3 cycles in the Atlantic are

not caused by dissolution, but rather by changes in CaCO_3 productivity or dilution with non-carbonate material^{12,14,17,18}. More recent work in the Atlantic, especially by CLIMAP¹⁹, has shown clearly that CaCO_3 cycles in the Atlantic are caused mainly by dissolution. The evidence for this is summarised briefly below.

Gardner¹³ showed that in the eastern equatorial Atlantic, cores from relatively shallow water (~3,000 m) have high CaCO_3 content (~80%) and that glacial-interglacial carbonate variations are small. However, with increasing water depth average CaCO_3 content decreases and glacial-interglacial amplitudes increase (Figs 3 and 4 in ref. 13). This systematic variation with depth cannot be attributed to changes in productivity because these cores are located close to each other. Carbonate dissolution is strongly controlled by depth (mostly because of pressure increase) and thus can better explain this variation. Gardner¹³ has also shown by material balance considerations that dilution alone cannot be responsible for the variation in CaCO_3 observed in this region. Following Gardner's calculation¹³, it has been shown that at least 60% of the CaCO_3 deposited during glacials in the eastern equatorial Atlantic must have dissolved²⁰. In addition, Gardner¹³ found that benthonic/planktonic foraminifera ratio and shell fragmentation indicate increased dissolution during the low carbonate glacial stages. Bé *et al.*²¹ showed a similar pattern in the western equatorial Atlantic. Glacial stages have low carbonate content, decreased coarse fraction, excessive fragmentation of planktonic foraminifera, absence of pteropods and increased benthonic/planktonic foraminifera ratio. Johnson *et al.*¹⁶, Prell and Hays²² and Thunell²³ found similar dissolution cycles in the southwestern Atlantic, Columbia Basin and the Gulf of Mexico, respectively. Therefore, although productivity changes and

Table 1 Comparison between the oxygen isotope composition of planktonic foraminifera from the uppermost sediment of two box cores in the North Atlantic Ocean, collected at 4,950 m and 4,500 m

Species	OC28-396 $\delta^{18}\text{O}$ (4,950 m)	OC28-395 $\delta^{18}\text{O}$ (4,500 m)	$\Delta\delta^{18}\text{O}$ (4,950-4,500 m)
<i>G. ruber</i>	-0.43	-0.71	+0.28
<i>G. truncatulinoides</i>	+0.98	+0.55	+0.43
<i>G. inflata</i>	+1.01	+0.74	+0.27
<i>G. hirsuta</i>	+1.23	+0.89	+0.34
<i>G. conglobatus</i>	+0.13	-0.20	+0.33
<i>G. sacculifer</i>	-0.55	-0.78	+0.23
<i>G. siphonifera</i>	+0.15	-0.06	+0.21
<i>O. universa</i>	-0.15	-0.20	+0.05
<i>P. obliquiloculata</i>	-0.12	+0.06	-0.18

Note that for all species except *P. obliquiloculata* $\delta^{18}\text{O}$ is higher in the deeper box core than in the shallow one. The difference in the isotopic composition is attributed to preferential dissolution of foraminifera that are isotopically lighter.

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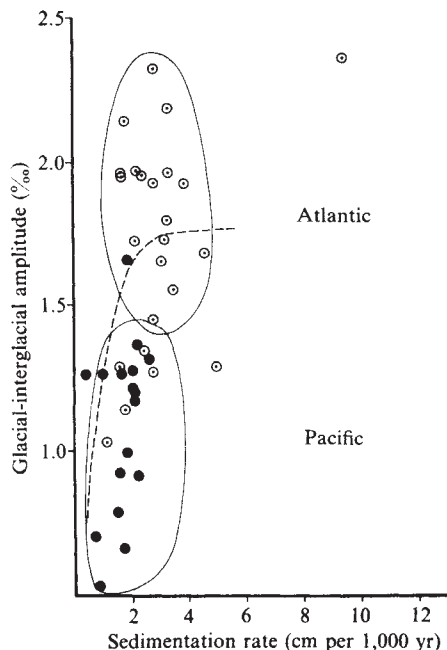


Fig. 1 The glacial-interglacial amplitude (GIA) of oxygen isotope curves in tests of planktonic foraminifera from sediment cores in the Atlantic (○) and Pacific (●) Oceans plotted against sedimentation rates in these cores. The isotopic composition is expressed in delta notation,

$$\delta^{18}\text{O} = \frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}}}{(^{18}\text{O}/^{16}\text{O})_{\text{standard}}} - 1 \times 1,000$$

Sources for the data and calculation of the amplitudes are discussed in Table 2. Note that the amplitudes in the Atlantic are significantly higher than amplitudes in the Pacific. The dashed line is the predicted GIA of a model suggested by Peng *et al.*⁵⁷ which accounts for bioturbation at different sedimentation rates. Most Atlantic amplitudes are higher, and Pacific amplitudes lower than the model predicts. The difference in the GIA between the two oceans is attributed to the effects of CaCO_3 dissolution cycles on the isotopic record.

dilution with non-carbonate material influenced carbonate content in certain areas, it seems that CaCO_3 cycles in both oceans are caused mainly by dissolution; they have similar frequencies as the glacials and interglacials; and the Atlantic and the Pacific are out of phase with respect to the timing of dissolution.

Today's oceans seem to be in agreement with the scheme suggested above. In the present interglacial the Atlantic is in a preservation mode, the CCD is roughly at 5–6 km and the saturation level ($\Omega = 1$) is at 4 km (ref. 24). The Pacific, on the other hand, is in a dissolution mode, its CCD is at 3–4 km and the saturation level ($\Omega = 1$) is at 1–2 km (ref. 24).

Effects of dissolution on the isotopic composition of planktonic foraminifera

Several authors have reported that Recent planktonic foraminifera recovered from the deep-ocean floor are isotopically heavier than their counterparts, recovered from shallower depths^{25–27}. It was suggested that this enrichment in ^{18}O is caused by selective dissolution^{25–28}. I have compared the isotopic composition of planktonic foraminifera recovered from the uppermost sediment of two box-cores from the north Atlantic (core OC28, 396 from 34°18' N, 57°12' W, in 4950 m water depth, and core OC28, 395 from 33°43' N, 57°27' W in 4,500 m water depth). The results, shown in Table 1, are in good agreement with earlier studies mentioned above^{25–27}. All species from the deeper box core with the exception of *Pulleniatina obliquiloculata* showed small but significant increase in $\delta^{18}\text{O}$ compared with the shallower box core. Visual observation of the

foraminifera in these box cores indeed indicates that the specimens from 4,500 m are better preserved than those from 4,950 m, suggesting that dissolution is the prime cause for the difference in the isotopic composition between these two samples²⁰.

Planktonic foraminiferal tests collected in plankton tows are thin and fragile²⁸. Their oxygen isotope composition is light (that is enriched in ^{16}O) and often in slight negative deviation from equilibrium^{29–31}, possibly caused by incorporation of isotopically light metabolic CO_2 which is enhanced by their symbiotic algae^{20,32}. As foraminifera sink through the water column, part of the population continues to calcify, more chambers are added and the tests become thicker^{20,33–37}. The isotopic composition of this part of the population is heavier because its skeleton is deposited in colder water and the activity of symbiotic algae ceases below the photic zone^{20,30,38}. Dissolution of the overall population tends to first remove the light-weight fragile individuals which are isotopically lighter²⁸. Consequently, the surviving population is isotopically heavier. This scheme which has recently been confirmed by comparison of planktonic foraminifera from plankton tows, sediment traps and sediments²⁰, explains how dissolution makes the isotopic composition of a species population heavier. It is now possible to examine the effect of CaCO_3 dissolution cycles on the isotopic record of the Pleistocene.

Effects of dissolution cycles on the isotopic record of the Pleistocene

When considering this problem, absolute values of $\delta^{18}\text{O}$ are of little interest because large variability exists between and within species due to their depth and geographic distribution. It should also be remembered that intercalibration between laboratories is not complete, especially because different treatments are used to remove the organic contaminants before isotopic analysis. For these reasons, I will consider here only the effects of dissolution on the isotopic glacial interglacial amplitude (GIA) which is comparable between species and between laboratories.

Most authors agree that the major factor controlling the GIA is ice volume in the Northern Hemisphere and its isotopic composition^{1–3,39–41}. Temperature, assumed to be the main factor by Emiliani^{5,6}, seems to be of secondary importance (see more recent reviews by Hecht⁴², Van Donk⁴³, and Shackleton⁴). Remarkable similarity exists in the shape and frequency content of $\delta^{18}\text{O}$ curves in the Atlantic and the Pacific Oceans. However, there is a large difference in the GIA between the two oceans^{1,5,6,41}. Figure 1 and Table 2 summarise most of the pertinent data on the GIA exhibited by planktonic foraminifera. Most Atlantic cores show an amplitude of ~1.9‰, whereas most Pacific cores show an amplitude of ~1.1‰. Emiliani^{5,6,44}, Imbrie *et al.*⁴⁰, Shackleton and Opdyke^{2,3}, and Berger and Gardner⁴¹, who tried to explain this difference, assumed that the Pacific responded only to the ice volume effect whereas in the Atlantic additional effects of temperature and evaporation/precipitation are superimposed on the ice-volume effect.

I suggest that most of the difference observed in the GIA between the Atlantic and the Pacific is caused by CaCO_3 dissolution cycles. Dissolution effects on the isotopic composition of a species population are quite small, perhaps in the range 0.2–0.4‰ (Table 1 and refs 25, 27). However, the timing of dissolution–preservation cycles within and between the two oceans can amplify the effects of dissolution on the GIA. Foraminifera in a well preserved core (even above the foraminiferal lysocline) are already partially dissolved⁴⁵, so the isotopic composition of such foraminifera is somewhat heavier than the original composition. Additional dissolution will make the isotopic composition even heavier, while better preservation will make the isotopic composition lighter and closer to the original.

In the Atlantic relatively heavy isotopic composition during glacials will become even heavier due to increased dissolution, and light isotopic composition during interglacials will become lighter because of preservation. Thus the total effect is ar.

Table 2 Glacial-interglacial amplitude (GIA) of oxygen isotope curves of planktonic foraminifera tests in deep-sea cores from the Atlantic and the Pacific Oceans

Core no.	Depth (m)	Location*	Species	$\delta^{18}\text{O}(\text{‰})$		GIA (‰)	Sedimentation rate (cm per 1,000 yr)
				Glacial	Interglacial		
Atlantic							
V28-14	1,855	NA	<i>G. pachyderma</i>	+4.64	+2.11	2.35	9.4
RC11-86	2,829	SA	<i>G. sacculifer</i>	+0.79	-0.55	1.34	2.5
RC12-294	3,308	SA	<i>G. bulloides</i>	+2.88	+0.96	1.92	2.8
V19-240	3,103	SA	<i>G. inflata</i>	+2.37	+0.72	1.65	3.1
V19-248	3,321	SA	<i>G. ruber</i>	+0.90	-0.13	1.03	1.2
V19-282	4,356	EA	<i>G. dutertrei</i>	+1.60	+0.31	1.29	5.0
V22-38	3,797	EA	<i>G. sacculifer</i>	+0.19	-0.95	1.14	1.8
V22-174	2,630	EA	<i>G. ruber</i>	+0.32	-1.23	1.55	3.3
V22-174	2,630	EA	<i>G. sacculifer</i>	+0.81	-0.92	1.73	3.3
A172-6	4,160	CR	<i>G. sacculifer</i>	+0.09	-1.87	1.96	3.3
A179-4	2,965	CR	<i>G. ruber</i>	-0.23	-2.04	1.81	1.8
A179-4	2,965	CR	<i>G. sacculifer</i>	+0.16	-1.80	1.96	1.8
A179-4	2,965	CR	<i>G. dubia</i>	+0.86	-1.04	1.90	1.8
A180-73	3,749	EA	<i>G. sacculifer</i>	+0.51	-1.44	1.95	1.7
234A	3,577	EA	<i>G. sacculifer</i>	+0.08	-1.76	1.84	1.2
234	3,577	EA	<i>G. sacculifer</i>	+0.15	-1.80	1.95	2.4
235A	4,560	EA	<i>G. sacculifer</i>	+0.24	-1.90	2.14	1.8
246	3,210	EA	<i>G. sacculifer</i>	+0.04	-1.64	1.68	4.6
280A	4,256	NA	<i>G. inflata</i>	+1.92	+0.63	1.29	1.6
P6304-8	3,927	CR	<i>G. sacculifer</i>	+0.63	-1.29	1.92	3.9
P6304-9	4,126	CR	<i>G. sacculifer</i>	+0.44	-1.35	1.79	3.3
A240-M1	4,180	CR	<i>G. sacculifer?</i>	+0.01	-1.71	1.72	2.2
A245 BR-C	2,968	CR	<i>G. ruber</i>	-0.30	-1.57	1.27	2.8
V12-122	2,800	CR	<i>G. sacculifer</i>	-0.01	-2.19	2.18	3.0
V12-122	2,800	CR	<i>G. sacculifer</i>	-0.15	-2.47	2.32	3.0
Pacific							
BNFC43 PG3	2,874	EP	<i>G. sacculifer</i>	-0.44	-1.80	1.36	1.1
RC8-94	3,074	SP	<i>G. sacculifer</i>	+0.81	+0.05	0.76	1.8
RC10-114	2,791	EP	<i>G. sacculifer</i>	-1.04	-1.66	0.62	0.9
RC11-210	4,420	EP	<i>G. sacculifer</i>	+0.16	-1.30	1.46	2.2
RC11-210	4,420	EP	<i>P. obliquiloculata</i>	+0.45	-0.92	1.37	2.2
RC11-230	3,259	EP	<i>G. sacculifer</i>	-0.06	-1.08	1.02	1.7
V21-33	3,726	EP	<i>G. sacculifer?</i>	+1.48	+0.18	1.30	2.2
V21-59	2,992	NP	<i>G. sacculifer</i>	-0.39	-1.19	0.80	0.8
V21-146	3,968	NP	<i>G. inflata</i>	+2.32	+1.01	1.31	2.1
V24-109	2,367	EP	<i>G. sacculifer</i>	-0.67	-2.08	1.41	2.7
V28-203	3,243	EP	<i>G. sacculifer</i>	-0.45	-1.72	1.27	2.2
V28-235	1,746	EP	<i>G. sacculifer</i>	-0.71	-1.80	1.09	1.9
V28-238	3,120	EP	<i>G. sacculifer</i>	-0.96	-1.97	1.01	2.3
V28-239	3,490	EP	<i>G. sacculifer</i>	-0.84	-1.72	0.88	1.6
WAH-8F2	4,308	EP	<i>G. obliquiloculata</i>	+0.73	-0.63	1.36	1.7
Y69-106P	2,870	EP	<i>G. sacculifer</i>	-0.20	-1.95	1.75	1.9
Y71-7-45P	3,096	EP	<i>G. sacculifer</i>	+0.24	-1.12	1.36	0.5

Data were selected from refs 2-6 and 40. Cores that did not have adequate sampling density or did not show the regular glacial-interglacial pattern were omitted. The amplitude was calculated by the difference between the highest and lowest $\delta^{18}\text{O}$ value in the uppermost section of each core, which represents the last glacial and the present interglacial. Sedimentation rate was calculated for the same core section assuming an age of 18,000 ys ago to the peak of the last glacial. Note that the GIA in the Atlantic is almost doubled compared to the Pacific.

* EA, Equatorial Atlantic; EP, Equatorial Pacific; NA, North Atlantic; NP, North Pacific; SA, South Atlantic; SP, South Pacific; CR, Caribbean.

increase in GIA (Fig. 2). In the Pacific the light isotopic composition during interglacials will become slightly heavier due to dissolution while the heavy isotopic composition during glacials will become lighter due to preservation. This will reduce the GIA in the Pacific (Fig. 2). If we assume that dissolution or preservation shifts the isotopic composition only by 0.2‰, the GIA in the Pacific may be reduced by 0.4‰, and amplified in the Atlantic by 0.4‰, thus the difference in the GIA between the two oceans may reach 0.8‰ which is quite close to the observed values (Table 2, Fig. 1).

The following studies by other investigators support my interpretation. Berger and Gardner⁴¹ have suggested that dissolution caused the extremely low GIA in the Pacific (0.2-0.8‰) reported by Emiliani⁵. Shackleton and Opdyke³ have also shown that within the Pacific, core V28-238 from 3,120 m recorded a larger GIA than core V28-239 from 3,450 m. The average difference in GIA between the two cores was 0.29‰. Shackleton and Opdyke² showed that the GIA recorded by benthonic foraminifera in core V28-238 is higher by 0.2‰ than that recorded by *Globigerinoides sacculifer*. They suggested that *G. sacculifer* migrated downwards during the interglacial to account for this observation. While this is not impossible, it is easier to explain this difference by dissolution that reduced the GIA recorded by *G. sacculifer*, but did not change the GIA

recorded by benthonic foraminifera which are more dissolution resistant.

Berger and Killingley²⁷ have recently compared the isotopic record of the past 19,000 yr in two western equatorial Pacific box cores from 1,597 m and 3,732 m. They showed that the isotopic composition of *P. obliquiloculata* (a dissolution-resistant species) was unaffected by dissolution, while the isotopic composition of *G. sacculifer* (which is dissolution susceptible) was significantly heavier in the deeper box core due to dissolution during the past 10,000 yr. Thus the GIA recorded by *G. sacculifer* in the deeper core is lower by 0.3‰ than the GIA recorded by *P. obliquiloculata*.

In the Atlantic, it is expected that dissolution resistant species will show a smaller GIA than dissolution susceptible species. Indeed, in the Caribbean core A-179-4 (from 2,965 m), Emiliani⁵ showed that while the dissolution susceptible species *G. ruber* and *G. sacculifer* had high GIA values of 2.05‰ and 1.94‰ respectively, the dissolution resistant species *G. dubia* (*dutertrei*) and *G. menardii* had GIA values of 1.37‰ and 0.75‰ respectively. This trend has been shown to persist through time by Lidz *et al.*⁴⁶ who found that *G. tumida*, *G. truncatulinoides* and *G. crassaformis* showed lower GIA values compared with dissolution susceptible species (*G. sacculifer*, *H. pelagica*, *O. universa* and *G. conglobatus*).

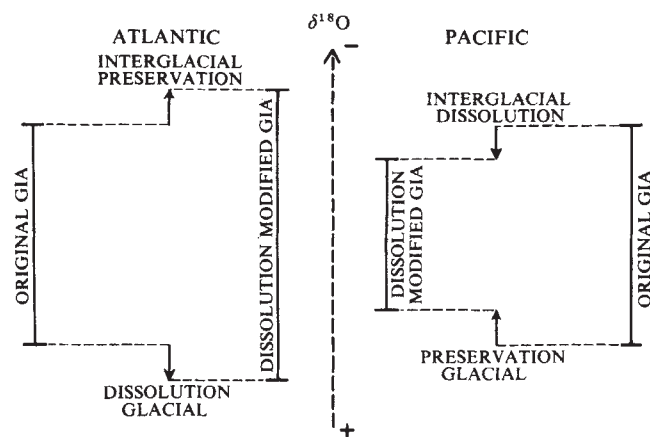


Fig. 2 Schematic presentation of modifications in the glacial-interglacial amplitude (GIA) of $\delta^{18}\text{O}$ curves caused by dissolution cycles in the Atlantic and the Pacific Oceans. The original GIA is that which would have been recorded in a well preserved core (above the lysocline) if dissolution-preservation cycles would not have existed. Note that CaCO_3 dissolution cycles amplify the GIA in the Atlantic while they reduce the GIA in the Pacific.

Discussion

The observations discussed above can be interpreted to show that processes other than dissolution were in operation. Emiliani^{5,6,44}, Shackleton and Opdyke², Lidz *et al.*⁴⁶, and Berger and Gardner⁴¹, suggested that the higher GIA in the Atlantic is a result of significant temperature fluctuations between the glacial and interglacials. Vertical migration of planktonic foraminifera combined with temperature changes between glacial and interglacials can potentially account for the differences between benthonic and planktonic foraminifera as well as the differences between shallow and deep dwelling planktonics. Quantitative estimates of palaeotemperatures derived from microfossil distribution in deep sea cores support the idea that small glacial-interglacial temperature fluctuations indeed occurred in the Atlantic^{44,47,48}. However, using the same quantitative techniques, other studies have shown that similar or even larger temperature fluctuations occurred in the Pacific⁴⁹. Therefore, micropalaeontologically derived palaeotemperatures cannot explain the difference in the GIA between the two oceans.

Recently Shackleton⁴ has shown that the GIA recorded by benthonic foraminifera is at least 1.65‰, and this value is believed to represent the change in $\delta^{18}\text{O}$ of ocean water due to ice accumulation and melting. In comparison, *G. sacculifer* in the Pacific shows an average GIA of 1.13‰ (based on 13 cores in Table 2). If this difference is interpreted in terms of temperature changes, it implies that *G. sacculifer* in the Pacific lived in warmer water during the glacial and colder water during the interglacial. While this is not impossible, it is more likely that the reduced GIA of *G. sacculifer* is caused by dissolution. If dissolution does reduce the GIA of planktonic foraminifera in the Pacific, it must also increase the GIA in the Atlantic. Indeed, *G. sacculifer* in the Atlantic shows an average GIA of 1.82‰ (based on 14 cores, Table 2). Therefore, there is no need to evoke cooling and warming of Atlantic surface water to explain the differences in the GIA between the two oceans.

Finally, effects of bioturbation on the isotopic record must be considered. It has been recognised since the pioneer work of Emiliani^{5,6} that bioturbation can blur the isotopic record and thus reduce the GIA^{4-6,50}. Because biogenic mixing affects only the upper few centimetres, cores with low accumulation rates are expected to show a lower GIA than those with high accumulation rates. Shackleton⁴ showed that this is indeed the case in a few selected cores from the Atlantic and the Pacific. However, in these studies no differentiation was made between planktonic and benthonic foraminifera. Peng *et al.*⁵¹ suggested a model to account for the effects of bioturbation on the GIA. Their model matches their data for benthonic foraminifera much better than it does for planktonic foraminifera (see Fig. 4 in ref. 51). Most of their data points for planktonic foraminifera are from the Pacific and they display a lower GIA than expected if bioturbation was the main factor in operation. Figure 1 shows the GIA versus sedimentation rate for all the data recently available for planktonic foraminifera (see also Table 2). In addition, the predicted GIA has been reproduced using the Peng *et al.*⁵¹ model. It is clear that most Pacific data points fall below the predicted GIA and most Atlantic data points fall above the predicted GIA. Thus, although the effects of bioturbation are certainly important, dissolution certainly adds a significant modification to the isotopic record that cannot be ignored. Perhaps when techniques to 'unmix' the past record^{51,52} are improved, better assessment of dissolution effects will be possible.

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Aminostratigraphy of UK Pleistocene deposits

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Ratios of D-alloisoleucine to L-isoleucine have been determined on freshwater molluscs from 13 interglacial sites in South East England and on marine molluscs and foraminifera from 10 Pleistocene and Upper Pliocene Crag sites in East Anglia. We can distinguish the Ipswichian, then either one or two interglacials that may be younger than the classical Hoxnian, then the Pleistocene Crag, then the Pliocene Coralline Crag.

AMINO ACID reactions derived from protein degradation in molluscan fossils have received considerable attention as a means of correlating and dating Pleistocene deposits¹⁻⁵. Although several reactions are potential chronological indices, in this study we have concentrated on the isoleucine epimerisation reaction. Over geological time, isoleucine, initially present in shell protein exclusively in the L configuration, epimerises to its non-protein diastereomer D-alloisoleucine. The proportion of the two acids, expressed as the allo/iso ratio, increases to an equilibrium value of 1.30 ± 0.05 . The rate of the epimerisation reaction is most sensitive to temperature; other potentially influential environmental parameters such as pH and leaching are effectively excluded by the integrity and natural buffering effects of the carbonate matrix. The activation energy for the isoleucine epimerisation reaction in carbonate fossils is ~ 29.0 kcal mol⁻¹ (ref. 6). This corresponds to an approximate doubling of the apparent age for every 4 °C temperature rise in the range between 0 and 20 °C. Consequently, absolute age assessment requires a precise estimate of the integrated thermal history since shell deposition for each site and an accurate kinetic model. It is, however, possible to circumvent the thermal term by analysing samples from a limited geographic region over which the temperature fluctuations, while unknown, can be assumed to have affected the region uniformly. In such a region, amino acid ratios may be used directly as correlation and relative age indices (aminostratigraphy⁷). We favour this approach as it requires a minimum of assumptions and yields the least ambiguous results. Another potential source of error results from the non-linear relationship between reaction rate and temperature. Samples lying close to the surface for a substantial portion of their burial history may yield anomalously high ratios due to their greater seasonal and diurnal temperature ranges. We prefer to analyse samples collected from at least 1 m depth.

Interglacial sites and amino acid analyses

For our work on interglacial deposits from the Thames estuary and adjacent sites we chose the freshwater mollusc *Corbicula fluminalis*. This shell is abundant in many English interglacial sites⁸. It does not occur in England today, but the genus is widely

distributed in the Old World and is currently invading the US⁹. Most of our sites were largely worked out in the last century, and most of our samples came from the collections in the British Museum (Natural History). We stress that the precise provenance of these samples is unknown. Without more historical research, the BMNH localities should be preceded by the words 'somewhere at'. However, this study was exploratory and for this purpose our samples have served very well.

From each site we have analysed three or more valves of *C. fluminalis* (when possible we select right valves to avoid analysing the same individual twice). Amino acid residues in both the free and total (free plus peptide-bound) fractions are determined on an automated ion-exchange amino acid analyser⁷. Monospecific samples were chosen to avoid problems with the species dependency of racemisation rates.

Table 1 shows the isoleucine epimerisation ratios in the total amino acid fraction for all *Corbicula* analysed. A certain amount of scatter in the amino acid ratios is expected; Miller and Hare⁹ suggest this should amount to less than 10–15% of the mean. The mean value is considered representative of the deposit for

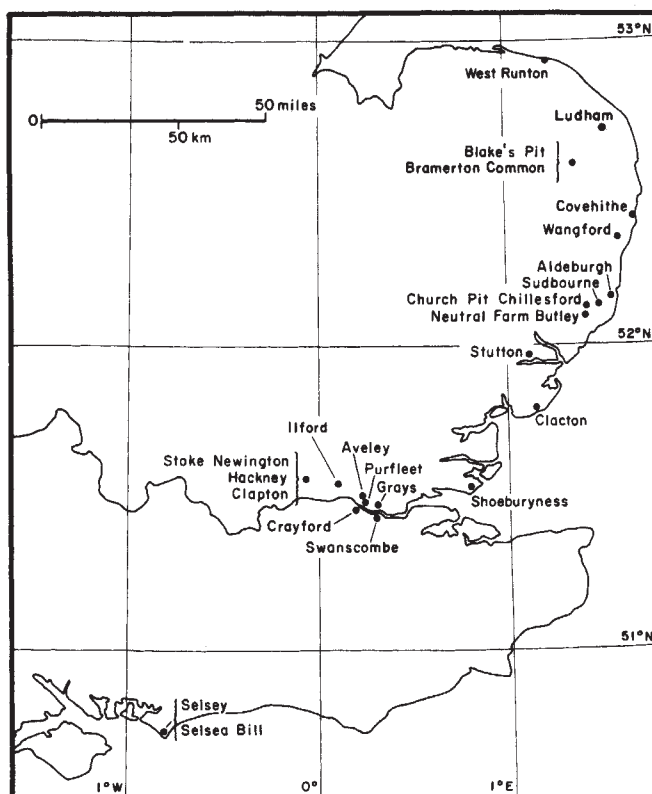


Fig. 1 South East England and East Anglia, showing sites studied.

Table 1 Isoleucine epimerisation in *Corbicula fluminalis*

Lab ID	Locality	Group	Isoleucine epimerisation				
			Indiv.	Lowest	$\bar{x}, \sigma$	$\sigma\%$	Asp/Glu*
812 A	Arkansas River† (USA)	1	0.020 0.021	0.020	0.020 ± 0.001	5	3.3 2.6
811 A	Aveley‡	2	0.20				3.5
B			0.16				4.6
C			0.20	0.16	0.19 ± 0.023	12	3.8
708 A	Stutton§	2	0.18				7.7
B			0.17				7.7
C			0.18				6.2
D			0.24				2.6
E			0.17				8.5
F			0.19				7.4
G			0.21				6.9
H			0.17	0.17	0.19 ± 0.024	13	6.8
701 A	Crayford (Norris's Pit)	2	0.18				8.4
B			0.21				7.6
C			0.26				4.4
D			0.22				—
E			0.19	0.18	0.21 ± 0.031	15	8.3
810 A	Clacton§	2	0.19	0.19	0.19	—	5.7
700 A	Ilford§ (Uphall Pit)	2	0.21				7.4
B			0.26				4.7
C			0.21				7.9
D			0.28				5.0
E			0.19	0.19	0.23 ± 0.038	17	7.6
707 A	Shoeburyness§	2	0.19				>3.5
B			0.26				3.9
C			0.25	0.19	0.23 ± 0.038	17	4.6
699 A	Selsey§	2 or 3	0.30				3.4
B			0.21				5.5
C			0.31				3.1
D-G			0.28	0.21	0.28 ± 0.045	16	3.9
705 A	Hackney§	2 or 3	0.24				3.7
B			0.26				4.7
C			0.31	0.24	0.27 ± 0.036	13	4.0
706 A	Clapton§	3	0.29				3.9
B			0.27				>3.4
C			0.29	0.27	0.28 ± 0.012	4	4.7
702 A	Grays§	3	0.29				3.8
B			0.28				5.0
C			0.33				3.9
D			0.34	0.28	0.31 ± 0.029	9	3.0
703 A	Swanscombe (Dierden's Pit)	3	0.30				6.6
B			0.29				5.8
C			0.29	0.29	0.30 ± 0.017	6	—
704 A	Stoke Newington§	3	0.29				3.5
B			0.30				3.4
C			0.34	0.29	0.31 ± 0.026	8	2.8
709 A	Purfleet¶ (shelly channel)	4	0.33				—
B			0.39				3.9
C			0.34	0.33	0.35 ± 0.032	9	3.3
710 A	Purfleet * (laminated beds)	4	0.34				4.0
B			0.34				4.1
C			0.37	0.34	0.36 ± 0.015	4	2.7
711 A	Wangford** (Hill Farm, Old Pit)	5	0.74				3.0
B			0.78				3.7
C			0.75	0.74	0.76 ± 0.021	4	4.1

Epimerisation was determined as the ratio of D-alloisoleucine to L-isoleucine in the total amino acid assemblage after conventional acid hydrolysis.

* We have noted a correlation between the ratio of aspartic acid to glutamic acid (Asp/Glu) and the isoleucine epimerisation ratio within shells of Group 2. *Corbicula* with low Asp/Glu values have high allo/iso ratios. In the Stutton collection analyses 708 D-H were made on different portions of a single valve. 708 D, the hinge area, yielded an allo/iso ratio substantially above the other portions (centre, central, anterior and posterior growing edges; E-H respectively). Similarly, the Asp/Glu ratio in 708 D is less than half the value of the other portions. The cause of such differences within a single valve is not readily apparent, but may be related to the calcification process. Weiner³⁷ has recently demonstrated a link between aspartic acid and calcium in mollusc shells. We have refrained from analysing the hinge area in other specimens to minimise the intravalve variation.

† *Corbicula* sp. from Russellville, Arkansas, provided by Dr L. R. Kraemer.

‡ Sample C from Aveley collected by Mr G. R. Ward of the Passmore Edwards Museum, from Hollin's (ref. 12) Unit 3. Samples A and B, from BMNH collections, are probably from the same unit.

§ Samples from BMNH collections, via Mr J. Cooper. The Stutton, Ilford, Shoeburyness, Grays, and probably the Clacton samples are from the Kennard collection, Selsey from the Davis collection, Hackney from the Evans collection and Stoke Newington from the Smith collection. The origin of the Clapton shells is uncertain.

|| Collector Dr M. P. Kerney. ¶ Collector J. T. H. * Collector Mr T. J. Allen. ** Collector Mr P. Cambridge.

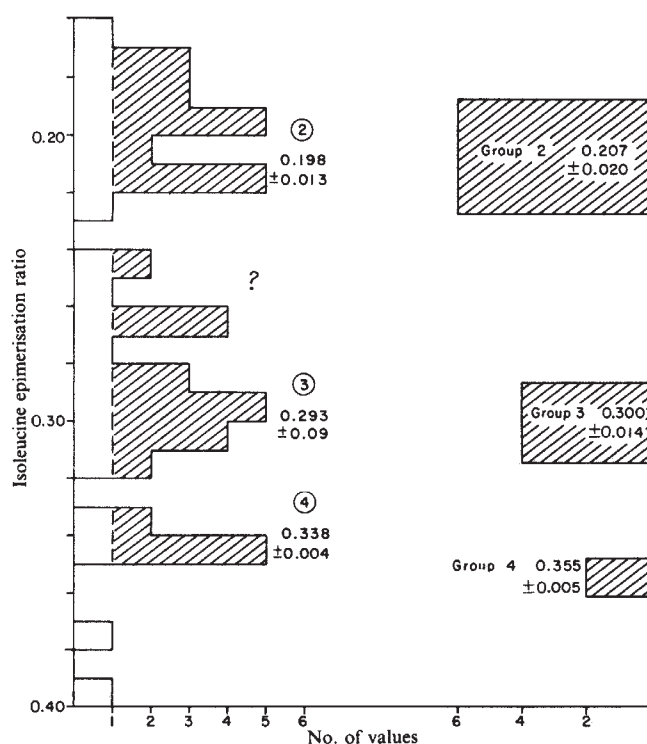


Fig. 2 Frequency distribution of individual isoleucine epimerisation (allo/iso) ratios in *Corbicula* from interglacial sites in SE England. Only ratios with more than one individual determination were considered significant in defining the three clusters on the left. Means and standard deviations from Groups 2-4 in Table 1 are on right for comparison

collections with a standard deviation of 15% or less. Greater deviations mean that one or more determinations are suspect. As in most Quaternary fluvial and shallow marine deposits, some samples may be reworked¹⁰. Hence, it may be more realistic to consider only the lowest ratio when comparing sites (Table 1). However, whether we consider the mean, or the lowest allo/iso ratios, five distinct groups are apparent in Table 1, with the only overlap being at Selsey and Hackney.

There is another, independent technique of evaluating the amino acid data. We can start with the assumption that the stratigraphy of most sites is not sufficiently documented to demonstrate the contemporaneity of each valve with a specific

stratum. Consequently we may simply have a collection of *Corbicula* from an unknown array of interglacial ages. In Fig. 2 we have plotted the distribution of individual allo/iso determinations between 0.15 and 0.40 (Groups 2, 3 and 4). The distribution is clearly non-random and three prominent clusters are apparent, with a possible additional cluster between allo/iso 0.24 and 0.26. The means and deviations for these clusters compare favourably with the means of Groups 2, 3 and 4 in Table 1 (see Fig. 2). Whether the allo/iso ratios are plotted by minimum or mean values for each collection, or simply as a composite of all values, five groups are clearly defined, with few ambiguous samples.

Amino acid groups and British Standard Stages

Group 1, a modern sample from Arkansas, is separated by a considerable interval from Group 2. Most of the Group 2 sites have been assigned by most workers to the last, Ipswichian, interglacial in the British Standard Stages¹¹. Comprehensive references to the sites at Aveley, Sutton, Crayford and Ilford are given in ref. 12. The location of the *Corbicula* site at Shoeburyness is uncertain¹³⁻¹⁵. The pollen at Selsey is typically Ipswichian¹⁶, and perhaps the higher ratios there represent a temperature effect on the shells, from either their southerly position or their shallow burial on the foreshore. However, note that Baden-Powell¹⁷ assigned some marine shells in this area to his 'Tyrrenian', last-but-one interglacial. The only anomalous result in Group 2 is from Clacton, which is generally assigned to the Hoxnian interglacial in the Standard Stages. Only a few *Corbicula* have been reported from Clacton¹⁸. The BMNH Kennard collection contains only a few valves, and we agreed to analyse one juvenile. Our result is so precisely Ipswichian that it is unlikely to represent a failure of our method. Either the collection is mixed, or perhaps the 3 km extent of the Clacton deposits^{18,19} includes a patch of Ipswichian material. In another attempt to relate the amino acid ratios to a Hoxnian pollen site, Cooper was able to supply one *Macoma* sp. marine shell from Selsey Bill and two *Macoma* from Clacton. In this case the Clacton shells were clearly older. Note from the Selsey ratios in Tables 1 and 2 that *Macoma* racemises more rapidly than *Corbicula*.

Most of our Group 3 sites have been assigned at some time to a last-but-one interglacial. However, none of them has yielded a diagnostic pollen diagram, and their assignment to the Hoxnian Stage is far from certain. The Hackney, Clapton and Stoke Newington samples are presumably all from the Hackney

Table 2 Isoleucine epimerisation in molluscs from Crag deposits

Unit	Site	No. of valves analysed	<i>Mya</i> sp. $\bar{x}$, σ	No. of valves analysed	<i>Macoma</i> sp. $\bar{x}$, σ
Ipswichian	Selsey Bill, Sussex*			1	0.33 (0.33)
Hoxnian	Clacton, Essex*			2	0.58 ± 0.03 (0.54)
Weybourne Crag	West Runton†	2	0.86 ± 0.028 (0.84)	2	0.90 ± 0.02 (0.83)
Norwich Crag Formation	Wangford near Hill farm†	3	0.89 ± 0.043 (0.84)		
	Covehithe†	3	0.89 ± 0.025 (0.84)		
	Blake's Pit†	3	0.96 ± 0.062 (0.91)	3	0.83 ± 0.044 (0.80)
	Bramerton Common†	2	1.02 ± 0.035 (0.99)	2	0.92 ± 0.02 (0.91)
	Church Pit‡ (Chillesford Crag)	2	1.08 ± (1.08)		
	Church Pit‡ (<i>Scrobicularia</i> Crag)	2	0.94 ± (0.94)	3	0.94 ± (0.94)
	Aldeburgh Pit†			3	1.02 ± 0.22 (0.83)
Red Crag Formation	Neutral Farm†‡	3	0.97 ± 0.098 (0.86)	3	0.71 ± 0.01 (0.67)
Coralline Crag	Sudbourne Park Pit‡	3	1.20 ± 0.02 (1.18)		

Epimerisation was measured as the ratio of D-alloisoleucine to L-isoleucine in the total acid hydrolysate. Values are mean ± s.d. of determinations on three individual valves when possible. Numbers in parentheses are lowest ratios.

* From BMNH collection, from J. Cooper.

† From P. Cambridge.

‡ Collector J. T. A.

Table 3 Isoleucine epimerisation in the foraminifera *Elphidiella hannai* from the Ludham Pilot Bore (Fig. 1)

Sample ID	Depth (m, O.D.)	Pollen zone	Foram. zone	Stage	Ratios
LP9	-11.7 to -13.1	L _p 4b/4c	L _r 6	Bav./Pre-Past.	0.79 0.81
LP17	-25.1 to -26.5	L _p 1b/2	L _r 3	Thur.-Lud. (early Norw. Crag)	0.81 0.83
LP20	-28.8 to -31.9	L _p 1b	L _r 2	Upper Lud. (earliest Norw. Crag)	0.90 1.06

Samples provided by B. M. Funnell, University of East Anglia, and prepared by A. R. Nelson, Dalhousie University. Each determination included several foraminifera tests. For a discussion of the stages here, see ref. 38.

London Borough sites exposed in the last century and reviewed by Wymer (ref. 20, p. 297–306). Hackney L. B. covers an area more than 3 km across, with elevations from 3 to above 30 m O.D., and it probably includes deposits of more than one age (ref. 20, pp. 317–319; ref. 27). The relatively low ratios from Hackney suggest the possibility of an interglacial stage or sub-stage intermediate between Groups 2 and 3 in age, represented perhaps by the cluster of ratios between 0.24 and 0.26 in Fig. 2. The Grays shells are separated in the BMNH collections from those from West Thurrock, so are almost certainly from Little Thurrock¹². Traditionally^{18,20,21–23}, the mammals at Little Thurrock have been assigned to a last-but-one interglacial, although West²⁴ and Hollin¹² thought that the polleniferous brickearth there was more likely to be Ipswichian than Hoxnian. The *Corbicula* from Little Thurrock must come from below 15 m O.D. (ref. 12). The *Corbicula* from our site at Dierden's Pit, just across the river at Swanscombe, are correlated by Kerney²⁵ with those reported from the Middle Gravels of the nearby Barnfield Pit, at about 27 m O.D. (ref. 20, pp. 332–354; ref. 26). *Corbicula* is notably absent from the underlying Lower Loam and Lower Gravel at Barnfield Pit²⁵, at an elevation intermediate between the Middle Gravels and Little Thurrock. This absence reinforces Hubbard's suggestion²⁷, that the Middle Gravels may be from a separate, younger interglacial than the Lower Loam and Lower Gravel. And the molluscs in the Lower Loam²⁵ and the archaeology from the Lower Gravel²⁰ resemble those at Clacton, where the pollen resembles that in the type site of the Hoxnian interglacial at Hoxne (Turner, in ref. 25).

Our Group 4 ratios come from the large interglacial site at Purfleet. Allen's²⁸ samples were collected 40 cm above the base of the laminated beds (Hollin's¹² Unit 2) in Greenlands north face. The ratios from the shelly channel (Unit 3) suggest that this is from the same interglacial. Hollin thought that the laminated beds were most probably Ipswichian, but very possibly older; and Allen thought that they might be from an interglacial between the Ipswichian and Hoxnian. The amino acid ratios suggest that they are at least as old as Dierden's Pit and the Middle Gravels at Swanscombe. This now fits in well with the conclusions of Palmer²⁹, that the archaeology from the overlying gravel and sand (Unit 4) at Purfleet resembles that in the Middle Gravels. Like that at Little Thurrock, the pollen in the laminated beds at Purfleet appears to be from the first half of an interglacial¹². There is some overlap in the ratios at these two sites, so perhaps this is the Group 3 interglacial; it is unlikely to be the Hoxnian, since *Corbicula* is not recorded in the first half of the interglacial at Clacton²⁵. And following the Clacton disappointment above, we have failed to locate any more *Corbicula* from a Hoxnian pollen site; the time relationship between our Groups 3 and 4 and the Hoxnian will have to be determined in some future campaign with some other species.

The Group 5 ratios come from the old pit at Hill Farm Wangford, in the Crag deposits, and provide a link with the marine, *Mya* sp. ratios below. Funnell and West (ref. 30, p. 253–254) discuss whether the 'Chillesford pollen assemblage' at Wangford is Pastonian (the interglacial before the Cromerian) or much older. Our results favour the latter.

Crag deposits of East Anglia

We report here on our efforts to stratify and correlate Crag deposits on the basis of the isoleucine epimerisation reaction in shells of the marine genera *Mya* and *Macoma* (Table 2). Our stratigraphic terminology follows Funnell and West³⁰. We are able to link the Crag deposits to the interglacial units by comparing allo/iso ratios in *Corbicula* and *Mya* from Wangford (Tables 1 and 2; 0.76 and 0.89 respectively). The *Mya* values are somewhat above those in *Corbicula*, suggesting a more rapid epimerisation in the former. We have little faith in the *Macoma* results from the Crag, first because in this case we have no link with *Corbicula*, and second because most *Macoma* yielded anomalously low total amino acid concentrations.

Individual allo/iso ratios in *Mya* from the Crag deposits range from 0.89 to 1.25. This result indicates that epimerisation has not reached equilibrium for most sites, in spite of their age. The *Mya* ratios from the Crag units define only two broad groups; a younger one that includes the Red Crag Formation, Norwich Crag Formation and Weybourne Crag, and a second, older one

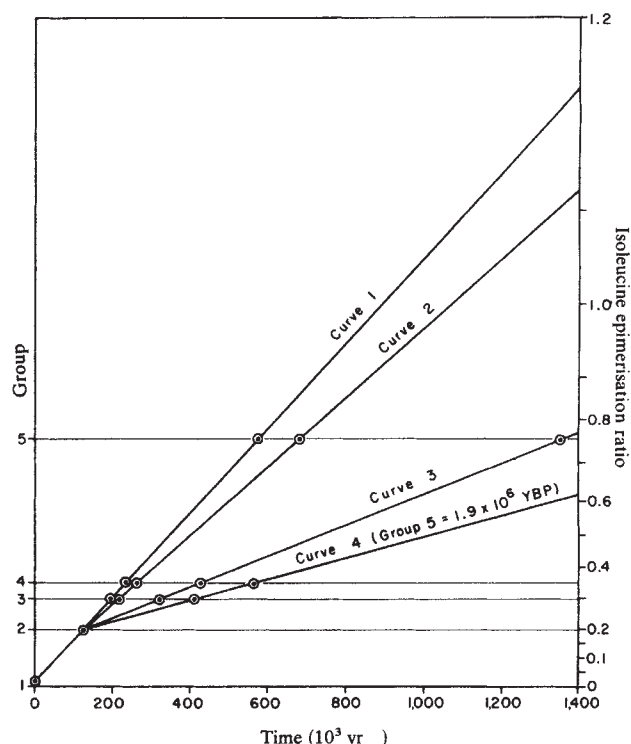


Fig. 3 Possible ages of Groups 3–5, assuming Group 2 is from 124,000 BP. Curve 1 assumes that reversible first order kinetics are followed exactly, Curve 2 that Group 3 is from 210,000 BP, Curve 3 that Group 3 is from 310,000 BP and Group 4 that Group 3 is from 410,000 BP.

consisting of the Coralline Crag. Shell fragments of *Mya* or *Arctica* collected from the Sudbourne Park Pit near Orford are from the lower shelly sands of the Coralline Crag. Allo/iso ratios are close to equilibrium, indicating that these Pliocene deposits are at the limit of the amino acid method for UK deposits. Within the younger group, average ratios range from 0.86 to 1.08. The Red Crag Formation is the oldest unit within this group and represents the basal deposits of the British Pleistocene succession. *Mya* from the Neutral Farm Pit of the Red Crag Formation have allo/iso ratios close to 1.0, significantly lower than those in the underlying Coralline Crag. Ratios in *Mya* from units within the Norwich Crag Formation range between 0.89 and 1.08, but are not always in correct stratigraphic order. Analyses of additional shell material should allow us to determine whether these discrepancies represent noise in the method or reflect reworking of shells from older units. The amino acid data suggest that the Weybourne Crag at West Runton is similar in age to the younger members of the Norwich Crag Formation.

Late in this study we were able to obtain foraminifera from the Ludham Pilot Bore in the Crag deposits. The epimerisation data (Table 3) are in the correct stratigraphic order and are similar to ratios in marine molluscs from correlative units.

Future work on the Crag of eastern England, especially if that work is concerned with the question of chrono-correlation, means analysing rather large numbers of samples. How large? If we wish to distinguish between the different Crag units, and if shells in these units differ in allo/iso ratios by 0.04, we can use our data to calculate that the number of runs required to discriminate between the units is equal to:

$$N = 4\sigma^2/L^2$$

where σ^2 is the variance of the population, and L is the limit we wish to apply (in our case, 0.04). If we set $\sigma^2 = \pm 0.1$ (a conservative figure) then we need 25 runs to be able to distinguish between two populations at the 95% confidence level. If $\sigma^2 = \pm 0.06$ then nine samples would be sufficient.

Discussion

The ratios above are generally in the correct chronostratigraphic order, and we discuss now how they may correlate with the worldwide marine oxygen isotope stratigraphy³¹ and with an absolute time scale³². In Group 2, the presumably tidal^{12,28} laminations at Crayford, the maritime herbs at Ilford, and the apparent level of the old floodplain at Aveley all suggest an early Ipswichian sea level of roughly 7 m (ref. 12: note that the possibility of the laminated beds at Little Thurrock and Purfleet being pre-Ipswichian was allowed for in that paper). In turn, this suggests a correlation with the worldwide high sea stand at 124,000 BP³³ and thereby with isotope Stage 5e³⁴. A U-series date of 125,000 BP has been obtained on a bone from Stutton³⁵.

On the assumption that Group 2 (mean ratio 0.207) dates from 124,000 BP, we can make some estimate of the ages of the older groups. However, conversion of allo/iso ratios into absolute ages is not straightforward. The isoleucine epimerisation reaction does not follow first-order reversible kinetics in carbonate fossils. Shell amino acids, initially bound in high molecular weight peptide chains, are slowly released in the free state over geological time but are retained within the carbonate matrix. The epimerisation rate differs for free and bound isoleucine [relative reaction rates as follows: terminal (C or N) » internal ≈ free]³⁶. Because the proportion of free isoleucine increases in older samples, there is a general reduction in the epimerisation rate with increasing sample age. First order kinetics may eventually be approximated, but at no point does the reaction rate increase. Hence, temporal extrapolations from younger (last interglacial) to older samples will yield minimum ages.

In Fig. 3, we have plotted the allo/iso ratios

$$\ln \left(\frac{1+D/L}{1-0.77(D/L)} \right) \text{ against time}^6$$

Curve 1 assumes shells in Group 2 are 124,000 yr old and that the isoleucine epimerisation reaction in *Corbicula* follows first order reversible kinetics exactly. The age predicted for Group 3 (190,000 BP) is a little low for the pre-Ipswichian interglacial Stage 7 (ref. 32). If we fix the age of Group 3 at 210,000 BP (roughly the mid-point of Stage 7), the age of Group 5 becomes ≥680,000 BP (curve 2). Fixing Group 3 at 310,000 BP (roughly the mid-point of the next interglacial, Stage 9), yields a Group 5 age of $\geq 1.35 \times 10^6$ BP (curve 3). Fixing Group 3 at 410,000 BP (an early estimate for the age of Stage 11), yields a Group 5 age of 1.9×10^6 BP (curve 4), which seems improbably old for the Norwich Crag.

We conclude, therefore, that our Group 3 probably belongs to isotope Stage 7 or 9. Unfortunately, we do not feel that the available geological evidence allows us to choose between these possibilities as yet. If we assign Group 3 ratios to Stage 7, extrapolation from Table 2 and Fig. 3 suggests an age for the younger Crag group of 0.7 to 1.2×10^6 BP and for the Coralline Crag of 1.9×10^6 BP. If we assign Group 3 to Stage 9, these ages become 1.0 to 1.8×10^6 BP and $\sim 3.0 \times 10^6$ BP. Especially valuable now would be a UK Pleistocene section with a recognisable palaeomagnetic reversal bracketed by shells.

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Direct expression in *Escherichia coli* of a DNA sequence coding for human growth hormone

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DNA coding for human growth hormone was constructed by using chemically synthesised DNA in conjunction with enzymatically prepared cDNA. This 'hybrid' gene was expressed in Escherichia coli under the control of the lac promoter. A polypeptide was produced having the size and immunological properties characteristic of mature human growth hormone.

HUMAN GROWTH HORMONE (HGH) is a protein of 191 amino acids which is synthesised in the anterior lobe of the pituitary. Growth in hypopituitary dwarfs, whose small stature is due to a deficiency of HGH, can be restored during childhood by administration of this hormone¹. In addition, HGH may prove effective in the treatment of a variety of ailments, including bone fractures, skin burns and bleeding ulcers². As growth hormone is species specific, human cadavers have been the only source of HGH.

The primary translation product of growth hormone mRNA is a precursor protein consisting of a signal peptide attached to the N-terminus of growth hormone³. Such signal or 'pre' sequences are characteristic of secreted proteins. In the case of rat growth hormone (RGH), DNA sequencing of cDNA prepared from RGH mRNA has identified the 26 amino acid residues of the pre-sequence⁴. From information derived from the cDNA sequence of human growth hormone⁵ we have designed and constructed a bacterial plasmid which instructs the synthesis of substantial quantities of mature HGH in a microbial cell.

HGH gene assembly strategy

The general approach used for the bacterial synthesis of HGH involves a combination of the cloning of the complementary DNA (cDNA) prepared from pituitary mRNA with the cloning of chemically synthesised DNA. The specific strategy was based on the known restriction endonuclease pattern of HGH cDNA⁵. *Hae*III sites are present in the 3' noncoding region and in the sequence coding for amino acids 23 and 24 of HGH. Treatment of double stranded (ds) HGH cDNA with *Hae*III gives a DNA fragment of 551 base pairs which includes coding sequences for amino acids 24–191 of HGH. We planned to clone this cDNA fragment and to clone separately a chemically synthesised DNA 'adaptor' fragment containing an ATG initiation codon and coding sequences for residues 1–23 of HGH. These two DNA fragments would be combined to form a synthetic–natural 'hybrid' gene. When inserted into a plasmid downstream from a suitable bacterial promoter and ribosome binding site, this gene could be expected to direct the synthesis of fMet-HGH. The fact that most bacterial proteins do not contain N-terminal methionine residues suggests that the fMet should be efficiently removed, resulting in the direct expression of HGH.

There are several advantages of this approach over conventional expression methods which utilise either chemically synthesised DNA or cDNA exclusively. The cDNA approach

has been used to express several fused proteins (β -lactamase–rat proinsulin⁶, β -lactamase–rat pre-growth hormone⁷ and β -galactosidase–chicken ovalbumin^{8,9}) in *Escherichia coli* which still retain antigenic activity, but which cannot be easily processed to give the natural gene product. However, the cloning of mouse dihydrofolate reductase cDNA into G-tailed, *Pst*I-cleaved pBR322 resulted in the expression of a protein which appeared to be dihydrofolate reductase¹⁰. This protein could have been derived from the proteolytic cleavage of a β -lactamase–dihydrofolate reductase fused protein or from the direct initiation of translation at the dihydrofolate reductase ATG (AUG) start codon. Neither of these possibilities could be relied on for HGH expression. *In vivo* proteolytic cleavage of a fused bacterially produced protein to give HGH is highly unlikely, and initiation of translation at an HGH start codon would be expected to yield the pre-hormone containing an extra 26 amino acids. The alternative approach of complete chemical synthesis of genes, although shown to be extremely effective for the bacterial production of the human peptides somatostatin¹¹ and insulin¹², would be very time consuming for a polypeptide as large as HGH.

Construction and cloning of plasmids containing HGH DNA sequences

The plan for the chemical synthesis of DNA designed to code for the first 24 amino acids of HGH is shown in Fig. 1a. To facilitate joining to HGH cDNA, the synthetic plan incorporates the same *Hae*III restriction site at amino acid residues 23 and 24 that is found in the cDNA. The 12 indicated deoxyoligonucleotides (Fig. 1a) were synthesised by the improved phosphotriester method essentially as described previously for insulin¹³. Figure 1b summarises the steps involved in the assembly and cloning of these DNA fragments in pBR322 (ref. 15). Many transformants of *E. coli* 294 (ref. 16) were obtained with plasmids containing *Eco*RI–*Hind*III inserts of approximately the desired size. The nucleotide sequences of three of these inserts were determined. One, pHGH3, had the expected DNA sequence. The other two had a deletion of the A·T base pair at the third nucleotide position for the codon of amino acid 5 (Pro).

The construction of the plasmid (pHGH31) containing the *Hae*III cDNA fragment of HGH is outlined in Fig. 2. Total poly(A)-mRNA from human pituitaries was used in the synthesis of ds-cDNA by RNA-dependent DNA polymerase (reverse transcriptase) and DNA polymerase I Klenow fragment. The cDNA was treated with *Hae*III restriction endonuclease and DNA of about 550 base pairs was purified by gel electrophoresis. This DNA fraction was then 'tailed' with deoxyC residues using terminal deoxynucleotidyl transferase¹⁰ and cloned in pBR322 DNA which had been cleaved with *Pst*I and extended with deoxyG residues. This procedure restores the *Hae*III restriction sites on the cDNA and regenerates *Pst*I sites at both ends of the insert. Approximately 200 transformants of *E. coli* χ 1776 (ref. 20) were obtained from 1 ng of C-tailed cDNA. These colonies were screened for the presence of HGH

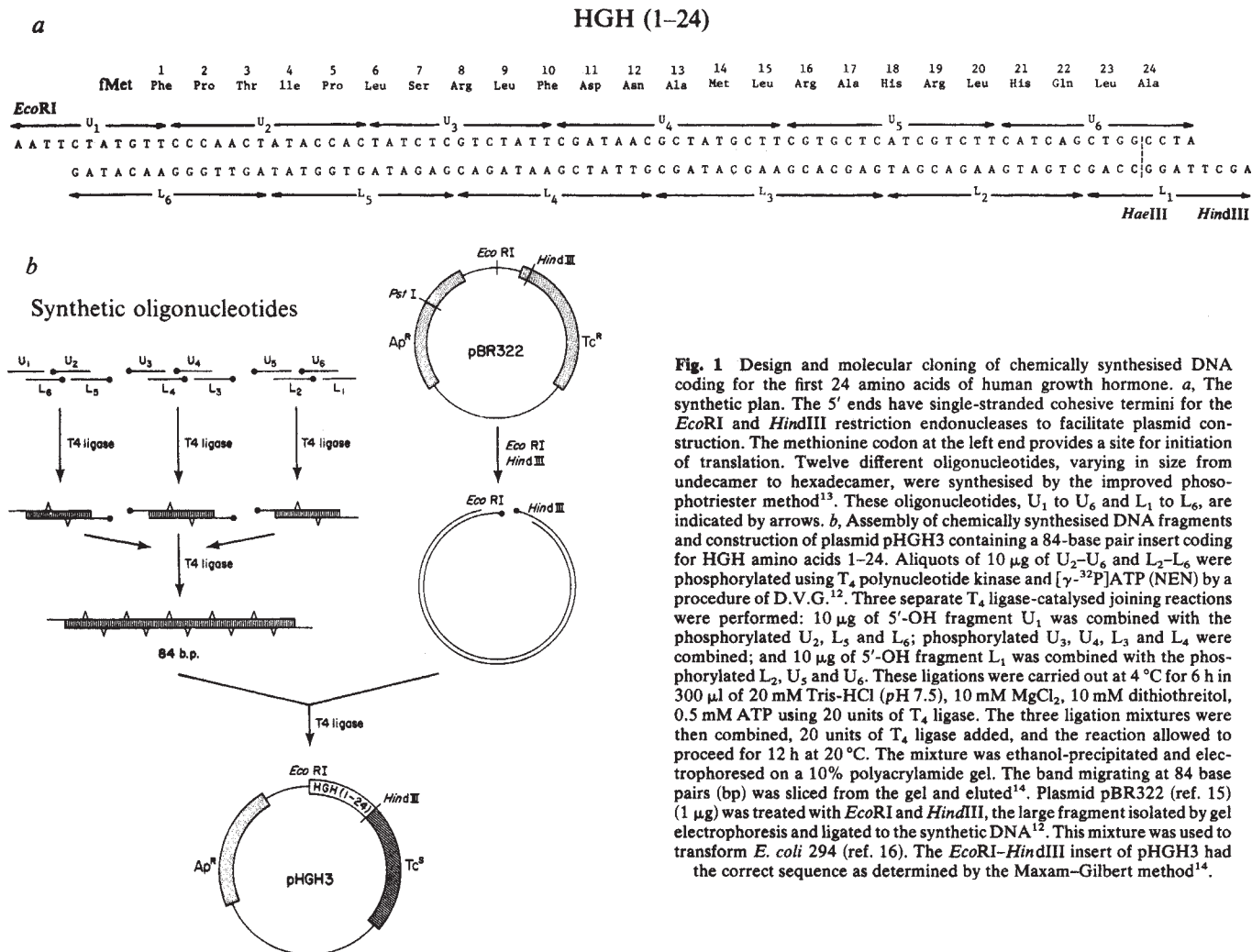


Fig. 1 Design and molecular cloning of chemically synthesised DNA coding for the first 24 amino acids of human growth hormone. **a**, The synthetic plan. The 5' ends have single-stranded cohesive termini for the *EcoRI* and *HindIII* restriction endonucleases to facilitate plasmid construction. The methionine codon at the left end provides a site for initiation of translation. Twelve different oligonucleotides, varying in size from undecamer to hexadecamer, were synthesised by the improved phosphotriester method¹³. These oligonucleotides, U_1 to U_6 and L_1 to L_6 , are indicated by arrows. **b**, Assembly of chemically synthesised DNA fragments and construction of plasmid pHGH3 containing a 84-base pair insert coding for HGH amino acids 1-24. Aliquots of 10 μ g of U_2 - U_6 and L_2 - L_6 were phosphorylated using T_4 polynucleotide kinase and [γ - 32 P]ATP (NEN) by a procedure of D.V.G.¹². Three separate T_4 ligase-catalysed joining reactions were performed: 10 μ g of 5'-OH fragment U_1 was combined with the phosphorylated U_2 , L_5 and L_6 ; phosphorylated U_3 , U_4 , L_3 and L_4 were combined; and 10 μ g of 5'-OH fragment L_1 was combined with the phosphorylated L_2 , U_5 and U_6 . These ligations were carried out at 4 °C for 6 h in 300 μ l of 20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 10 mM dithiothreitol, 0.5 mM ATP using 20 units of T_4 ligase. The three ligation mixtures were then combined, 20 units of T_4 ligase added, and the reaction allowed to proceed for 12 h at 20 °C. The mixture was ethanol-precipitated and electrophoresed on a 10% polyacrylamide gel. The band migrating at 84 base pairs (bp) was sliced from the gel and eluted¹⁴. Plasmid pBR322 (ref. 15) (1 μ g) was treated with *EcoRI* and *HindIII*, the large fragment isolated by gel electrophoresis and ligated to the synthetic DNA¹². This mixture was used to transform *E. coli* 294 (ref. 16). The *EcoRI*-*HindIII* insert of pHGH3 had the correct sequence as determined by the Maxam-Gilbert method¹⁴.

sequences by the Grunstein-Hogness hybridisation procedure²¹ using 32 P-labelled human chorionic somatomammotropin cDNA²² as probe. Seven colonies hybridised specifically with the probe, which is nearly identical to HGH in nucleotide sequence⁵. Three had identical *HaeIII* inserts of approximately 550 base pairs containing *SmaI*, *BglII*, *PstI* and *PvuII* sites, all characteristic of HGH cDNA⁵. Sequence analysis of the insert from one of these clones, pHGH31 (Fig. 3) gave only one discrepancy (CUG versus CUA codon for leucine 101) when compared with the previously determined cDNA sequence⁵. The amino acid sequence derived from the cDNA sequence differs from the published amino acid sequences of Li²³ at positions 74 (Glu versus Gln), 107 (Asp versus Asn) and 109 (Asn versus Asp). Although we cannot rule out post-transcriptional modification, the discrepancies probably stem from shortcomings of earlier protein sequencing methods in the identification of amidated amino acids.

Construction and cloning of HGH expression plasmids

To express directly HGH in *E. coli*, a plasmid (pGH6) having two *lac* promoters was constructed as follows. A 285-base pair *EcoRI* fragment containing two 95-base pair UV-5 *lac* promoter fragments separated by a 95-base pair heterologous DNA fragment was isolated from the plasmid pKB268 (ref. 24) and inserted into pBR322 at the *EcoRI* site. A plasmid (pGH1) was isolated with the promoter orientated to initiate transcription in the direction of the tetracycline resistance (*tc*^R) gene. The *EcoRI* site distal to the *tc*^R gene was destroyed by a previously outlined procedure¹¹.

Figure 4 reviews the steps involved in assembling the two separately cloned HGH DNA fragments. Treatment of pHGH3

with *EcoRI* and *HaeIII* gives a 77-base pair fragment with an *EcoRI* cohesive end and a *HaeIII* blunt end (also see Fig. 1a). This fragment, which codes for HGH amino acids 1-23, was isolated from a polyacrylamide gel. The plasmid pHGH31 was cleaved with *HaeIII* and the 55-base pair HGH cDNA fragment isolated by gel electrophoresis. *XmaI* treatment of this fragment yielded a 512-base pair fragment coding for amino acids 24-191 of HGH with one *HaeIII* blunt end and one *XmaI* sticky end. The ligation of these two fragments resulted in the formation of high molecular weight DNA. Treatment with *SmaI* (which recognises the same sequence as *XmaI* but leave flush ends) and *EcoRI* converted the ligation products to three distinct fragments: a 154-base pair dimer of the 77-base pair DNA fragment, a 1,024-base pair dimer of the 512-base pair fragment, and the desired 591-base pair DNA coding for the entire HGH sequence. This latter fragment was purified by gel electrophoresis and inserted into pGH6 between the *EcoRI* and the *S*₁ nuclease-treated *HindIII* sites. Following transformation of χ 1776, the expression plasmid pHGH107 was identified by colony screening and restriction analysis as described in Fig. 3. The HGH DNA sequence of pHGH107 was verified by the Maxam-Gilbert procedure¹⁴.

In our plan to express HGH two base pairs were included between the *EcoRI* AATT 'sticky' end and the ATG codon of the chemically synthesised portion of the HGH coding sequence (see Fig. 1). Therefore, in the plasmid pHGH107, 11 base pairs separate the *lac* AGGA ribosome binding site²⁶ and the ATG translational start for HGH:

5'...AGGAAACAGAATTCTATG...
3'...TCCTTTGTCTTAAGATAC...

In the naturally occurring *lac* system the separation between

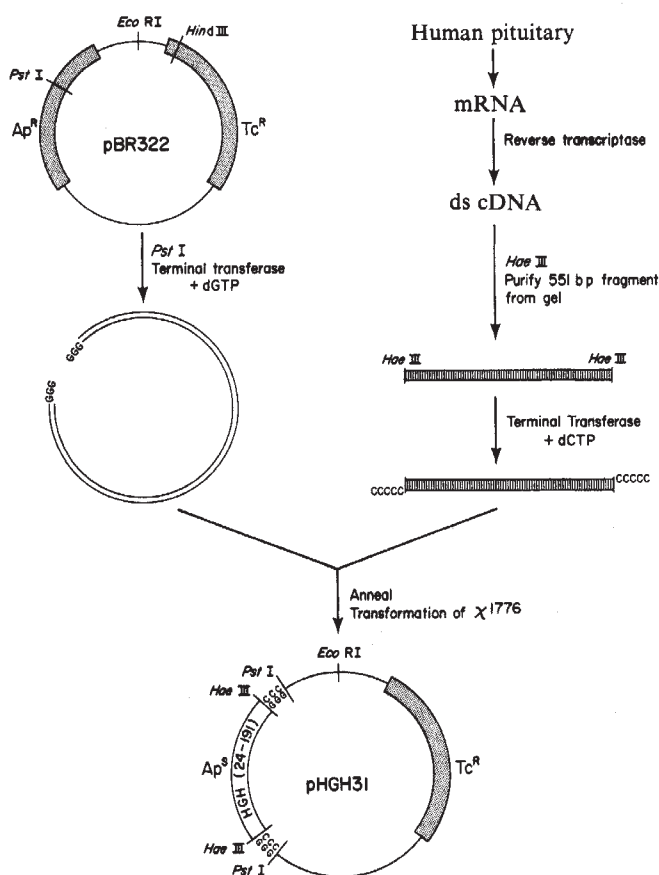


Fig. 2 Construction of a plasmid (pHGH31) containing coding sequences for amino acids 24–191 of human growth hormone. Poly(A)-mRNA was prepared from human pituitaries by a published procedure¹⁷. ds-cDNA (1.5 μg) was prepared from 5 μg of this RNA essentially as described by Wickens *et al.*¹⁸, except that DNA polymerase 'Klenow fragment'¹⁹ was substituted for DNA polymerase I in the second strand synthesis. cDNA (90 ng) was treated with *Hae*III, electrophoresed on an 8% polyacrylamide gel, and the region around 550 base pairs eluted. Approximately 1 ng of cDNA was obtained. Terminal deoxynucleotidyl transferase (TdT) was used to add approximately 20 dC residues per 3' terminus¹⁰. Annealing of the dC-tailed ds-cDNA with the dG-tailed vector DNA (60 ng) was performed in 130 μl of 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.25 mM EDTA. The mixture was heated to 70 °C, allowed to cool slowly to 37 °C (12 h), then to 20 °C (6 h), before being used to transform χ1776 (ref. 20) by a published procedure⁴.

ribosome binding site and initiation codon is 7 base pairs. The exact *lac* sequence in this region was restored as follows. The plasmid was cleaved with *Eco*RI, treated with *S*₁ nuclease to digest the single stranded ends, and reclosed by blunt-ended ligation. The plasmids from three individual transformants of χ1776 were analysed. In all instances the *Eco*RI site had been removed and a new *Alu*I site created in the plasmid (pHGH107–1).



This structure, in which the ATG codon is now seven nucleotides downstream from the ribosome binding site, might be expected to increase the efficiency with which HGH mRNA is translated.

HGH radioimmune activity in bacterial extracts

Extracts of *E. coli* χ1776 containing the expression plasmids were tested for HGH expression by a direct radioimmunoassay (RIA) (Table 1). Easily detectable amounts of HGH were produced by bacteria containing pHGH107 or the derivative of pHGH107 lacking the *Eco*RI site (pHGH107–1). Contrary to

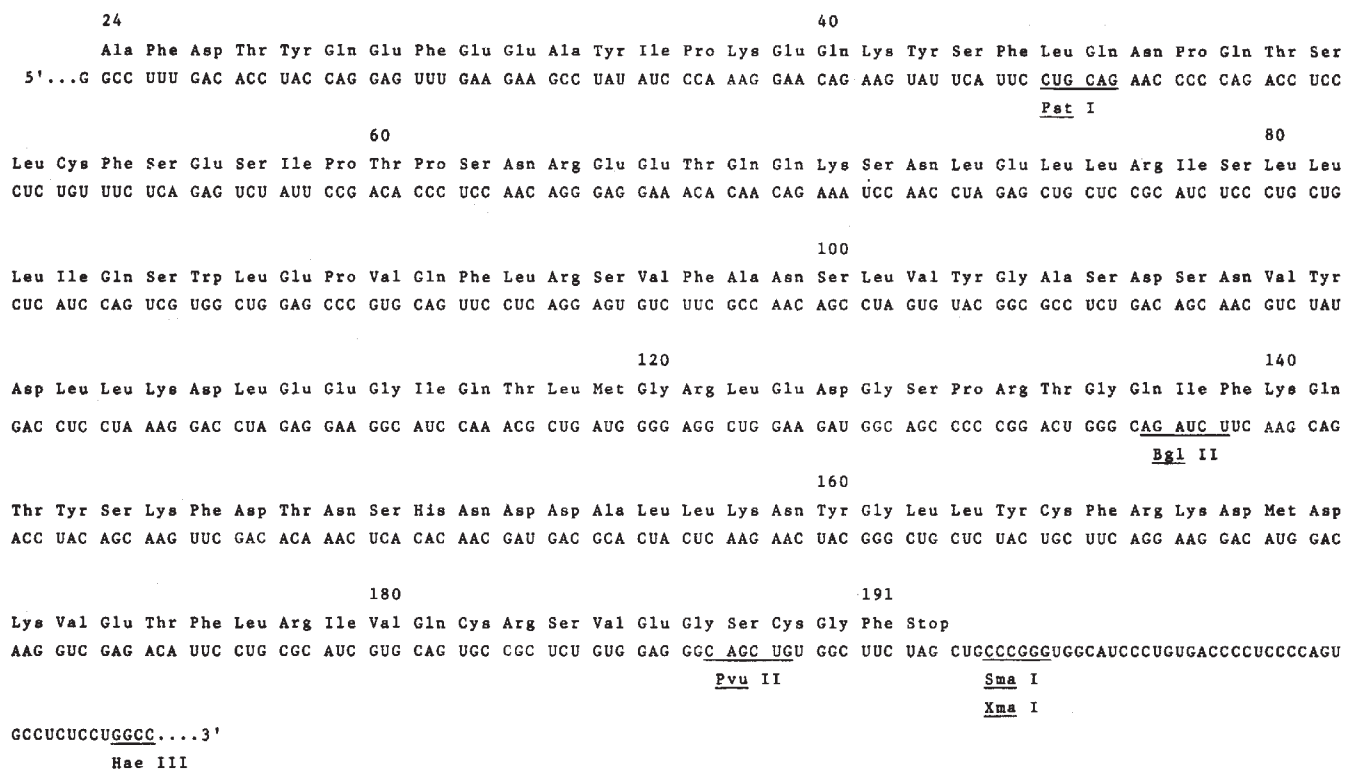


Fig. 3 The amino acid and mRNA sequences of HGH as determined by DNA sequencing¹⁴ of pHGH31. The nucleotide sequence from the *Sma*I site to the *Hae*III site in the 3' noncoding region is from ref. 5, and was not determined here.

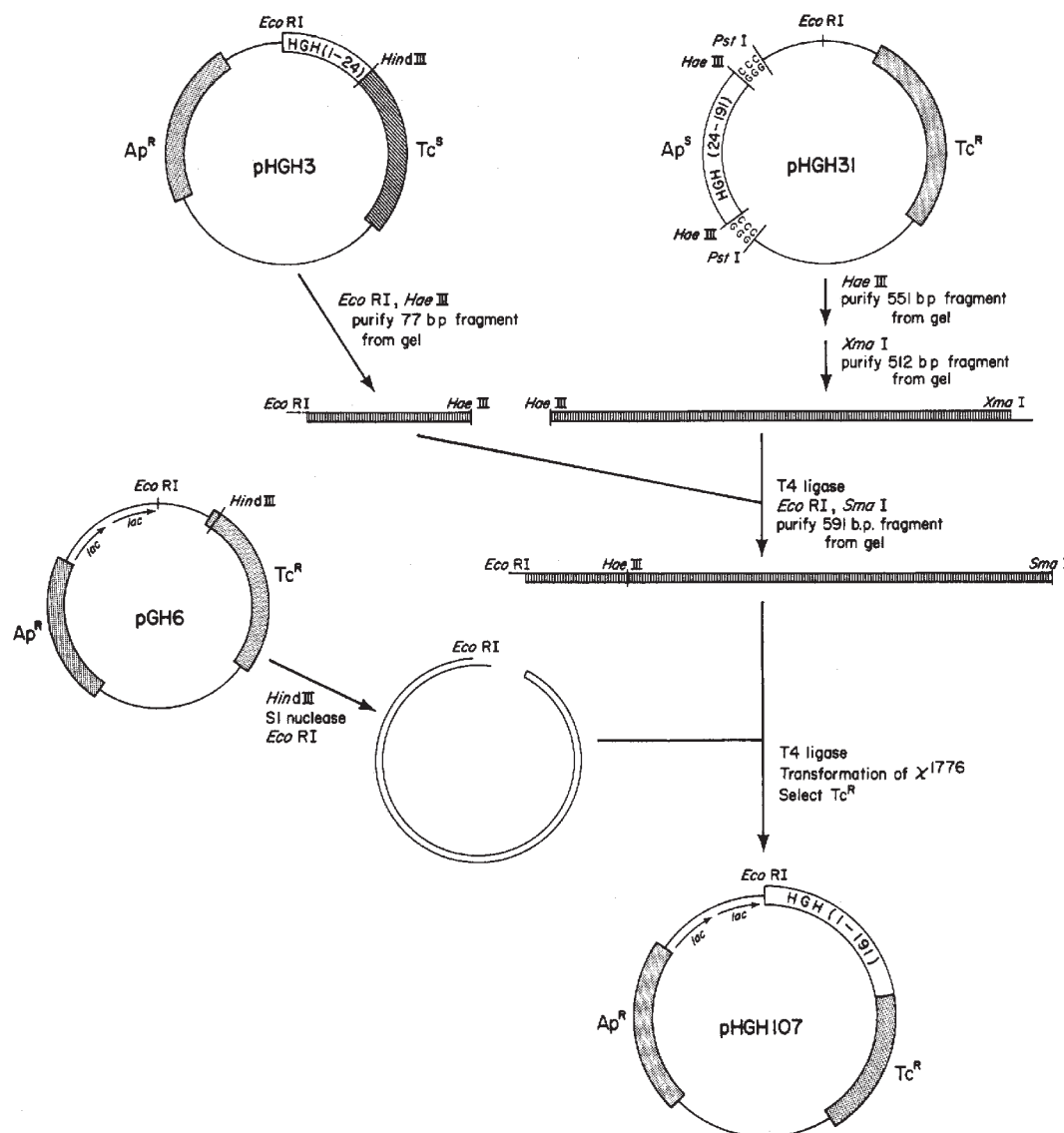


Fig. 4 Construction of a plasmid (pGH107) for the bacterial expression of human growth hormone. pHGH3 (10 μ g) was cleaved with *EcoRI* and *HaeIII* restriction endonucleases and the 77-base pair fragment containing coding sequences for HGH amino acids 1–23 was isolated from an 8% polyacrylamide gel. The plasmid pHGH31 (~5 μ g) was cleaved with *HaeIII*. The 551-base pair HGH sequence and a co-migrating 540-base pair *HaeIII* fragment of pBR322 were purified by gel electrophoresis. Subsequent treatment with *XmaI* cleaved only the HGH sequence, removing 39 base pairs from the 3' noncoding region. The resulting 512-base pair fragment was purified from the 540-base pair pBR322 *HaeIII* piece by electrophoresis on a 6% polyacrylamide gel. Samples of 0.3 μ g of the 77-base pair *EcoRI*–*HaeIII* fragment and 0.3 μ g of the 512-base pair *HaeIII*–*XmaI* fragment were polymerised with *T₄* DNA ligase in a 16- μ l reaction for 14 h at 4 $^{\circ}$ C. The mixture was heated at 70 $^{\circ}$ C for 5 min to inactivate the ligase, then treated with *EcoRI* (to cleave fragments which had dimerised through their *EcoRI* sites) and with *SmaI* (to cleave *XmaI* dimers), yielding a 591-base pair fragment with an *EcoRI* 'sticky' end and a *SmaI* 'blunt' end. After purification on a 6% polyacrylamide gel, approximately 30 ng of this fragment were obtained. The expression plasmid pGH6 containing tandem *lac* UV-5 promoters, was treated successively with *HindIII*, nuclease *S₁*, and *EcoRI* and

purified by gel electrophoresis. This removes most of the promoter sequence of the *tc^R* gene, but leaves the structural gene intact (25). 50 ng of the resulting vector, which had one *EcoRI* sticky end and one blunt end, was ligated to 10 ng of the 591-base pair HGH DNA. The ligation mixture was used to transform χ 1776. Colonies were selected for growth on tetracycline (12.5 g ml⁻¹). Since the *tc^R* promoter is no longer functional, tetracycline resistance is dependent on transcription from the *lac* promoters reading through the HGH gene sequence into the *tc^R* gene. Approximately 400 transformants were obtained. Colony screening by filter hybridisation²² identified 12 colonies containing HGH sequences. The plasmids isolated from three of these colonies gave the restriction patterns expected for the correctly assembled gene when cleaved with *HaeIII*, *PvuII* and *PstI*. The DNA sequence of one clone, pHGH107, was determined.

our expectations, the introduction of four extra base pairs of DNA into the *lac* sequence in the region between the ribosome binding site and initiation codon results in increased HGH synthesis.

To verify that HGH expression is under *lac* operon control the plasmid pHGH107 was transformed into the *E. coli* K12 strain D1210, an *i^Q* derivative of *E. coli* K12 HB101 which overproduces *lac* repressor¹². The data in Table 1 demonstrate that repressor effectively blocks HGH production and that this effect can be reversed by the addition of the inducer isopropyl- β -D-thiogalactoside.

Identification of HGH from bacterial extracts

Extracts of *E. coli* K12 strain RV308 (ref. 27) containing either pHGH107 or pBR322 were electrophoresed on SDS-polyacrylamide gels. The resulting protein patterns show one clear difference (Fig. 5a)—an additional protein, which co-migrates with HGH isolated from pituitaries, appears in the RV308/pHGH107 extract. To achieve partial purification of HGH, an extract of a 1-litre culture of χ 1776/pHGH107 was prepared and fractionated as described in Fig. 5 legend. The polyacrylamide gel electrophoresis pattern of this partially

Table 1 Radioimmunoassay of HGH in extracts from bacteria containing expression plasmids

Strain	Cell density (cells per ml)	HGH by RIA (μ g per ml)	HGH copies per cell
χ 1776/pHGH107	3.69×10^8	2.4	186,000
	1×10^9	1.4	39,000
χ 1776/pHGH107-1	3.6×10^8	1.5	116,000
	1×10^9	0.5	14,000
χ 1776/pBR322	3.6×10^8	0	0
D1210/pHGH107	3.8×10^8	2×10^{-4}	15
D1210/pHGH107 (IPTG)	3.8×10^8	1.0	75,000

E. coli strains containing the appropriate plasmid were grown to the indicated cell density and collected by centrifugation. Stationary phase cultures of χ 1776 contain 10^9 cells per ml. The cell pellet was resuspended in 55 μ l of 50 mM Tris-HCl (pH 8), 50 mM EDTA, 15% sucrose, 1 mg ml⁻¹ lysozyme, 0.025% lithium dodecylsulphate. After 30 min at 0 $^{\circ}$ C, 10 μ l of 150 mM Tris-HCl (pH 7.5), 280 mM MgCl₂, 4 mM CaCl₂ and 1 μ g of DNase I were added. The mixture was centrifuged for 15 min at 12,000g. Serial dilutions of the supernatants were analysed by radioimmunoassay using the Phadebas HGH PRIST kit (Pharmacia). Values are the average of three independent experiments expressed as μ g per ml of cell culture. With HGH standard, the detection limit in 1 ml of culture was 0.02 ng. No activity was found in extracts of χ 1776/pBR322. Where indicated, isopropyl- β -D-thiogalactoside (IPTG) was added to the cell culture at a concentration of 2 mM.

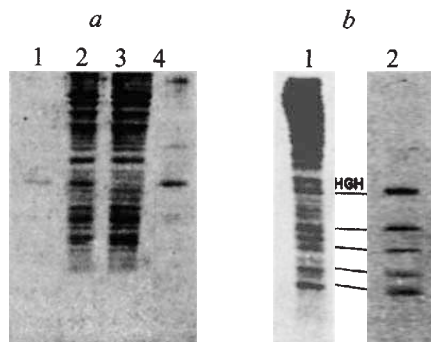


Fig. 5 Identification of HGH produced in bacteria by SDS-polyacrylamide gel electrophoresis. *a*, Protein patterns of crude extracts and partially purified HGH stained with Coomassie brilliant blue. Slot 1 contains 0.5 μ g of pituitary HGH standard (Kabi), slot 2 contains a cell lysate of RV308/pHGH107, slot 3 contains a cell lysate of RV308/pBR322, slot 4 contains partially purified HGH isolated from χ 1776/pHGH107. The samples were separated on a 15% polyacrylamide slab gel using the buffer system of Maizel²⁸ with the addition of 6 M urea. Crude lysates were prepared by growing cells in LB with 5 μ g ml⁻¹ tetracycline followed by lysis in 2% SDS, 1% β -mercaptoethanol. The lysates were precipitated with 10 volumes of cold acetone and the pellets were redissolved in SDS sample buffer for use in gel electrophoresis. The partially purified HGH was prepared from a stationary phase culture of χ 1776/pHGH107. Cells were collected and resuspended in 1/50 of their original volume in 30 mM potassium phosphate (pH 7.0) containing 0.05 M NaCl and lysed by sonication. Polyethylenimine (Miles, Polymix-P) was added to 0.2%. After centrifugation for 1 h at 100,000g, ammonium sulphate was added to the supernatant to 60% saturation. The ammonium sulphate pellet was dissolved in 2 ml 10 mM potassium phosphate (pH 7.0), 0.5 M NaCl and chromatographed on a Sephacryl S-200 column (2.0 $\times$ 50 cm) equilibrated in the same buffer. The radioimmune active peak (assayed with a Pharmacia Phadebus HGH kit) was pooled, the protein concentrated by ammonium sulphate precipitation, the pellet redissolved in 1/10 volume of buffer, and the solution dialysed against 10 mM potassium phosphate (pH 7.0), 0.5 M NaCl. Precipitated material was removed by centrifugation resulting in a HGH preparation of approximately 25% purity. *b*, Autoradiograms of ³⁵S-labelled extracts of RV308/pHGH107. Slot 1 contains a total lysate of RV308/pHGH107 labelled with H₂³⁵SO₄. Slot 2 contains a ³⁵S-labelled extract of RV308/pHGH107 precipitated with α -HGH antiserum. The major (top) band co-migrates with unlabelled HGH standard (not shown). Cultures (1 ml) of RV308/pHGH107 were grown to A₅₅₀ = 1 in low sulphur medium²⁹ containing 0.2 mCi ml⁻¹ H₂³⁵SO₄, chased with 10 mM MgSO₄ for 5 min, collected and lysed using Triton X-100 and lysozyme³⁰. Following DNase and RNase treatment²⁹, the lysate was mixed with a 10-fold excess of unlabelled RV308/pBR322 extract and diluted 1:1 into Triton immunoprecipitation buffer (0.15 M NaCl, 1% Triton X-100, 0.05 M Tris-HCl pH 7.5). Twenty μ l of α -HGH antiserum (Kabi) was added per ml of original culture and the reaction was incubated for 12 h at 4 °C. The mixture was centrifuged and the supernatant incubated for 2 h with formaldehyde fixed *Staphylococcus* cells³¹, filtered on 0.45- μ m Nucleopore polycarbonate filters, washed with Triton immunoprecipitation buffer and extracted with SDS sample buffer²⁸. The samples were run on a 15% slab gel containing urea and SDS as described above.

purified sample is shown in Fig. 5*a*, slot 4. The most prominent band co-migrates with HGH standard and its relative intensity is consistent with the 25% purity calculated from radioimmune activity.

In an independent attempt to confirm that the antigenically active component in the bacterial extracts co-migrates with

HGH in SDS gels, we precipitated extracts from cells labelled with ³⁵S with antiserum prepared against HGH and separated the precipitated proteins by polyacrylamide gel electrophoresis. The autoradiogram of the immunoprecipitated material (Fig. 5*b*, slot 2) shows a major band, with electrophoretic mobility identical to that of HGH, as well as four smaller bands. All five of these bands are present in the unfractionated ³⁵S-labelled lysate (slot 1), whereas only the band with the same mobility as HGH can be seen in unlabelled extracts of cells grown in LB broth (Fig. 5*a*, slot 2). All five immune precipitated bands can be competed out by excess cold HGH (data not shown) and are thus antigenically related. This suggests that the four smaller HGH-related products observed in minimal media may be due to proteolytic degradation.

Additional evidence of proteolytic degradation of HGH is presented in Table 1. The levels of HGH in extracts from strains containing pHGH107 or pHGH107-1 were considerably higher in log-phase cultures than in stationary-phase cultures. Strains transformed with several other plasmids whose products are under *lac* promoter control show no comparable decrease of gene expression as a function of growth phase (unpublished results from this laboratory). It thus seems that there is some turnover of HGH in bacterial cells although we cannot rule out other explanations for the four smaller HGH-related products in the ³⁵S-labelled extracts such as premature termination of translation.

Despite the observed instability of HGH in certain growth conditions, the molecule seems to be relatively stable in log-phase cultures grown in rich media. In these conditions the amount of HGH in the cells (186,000 monomers per cell, Table 1) compares favourably with the expression level of other cloned genes using the same promoter in optimised conditions³².

Conclusion

Using a novel combination of chemically synthesised DNA and cDNA, a recombinant *E. coli* strain has been constructed which produces HGH in large amounts. This is the first time that a human polypeptide has been directly expressed in *E. coli* in a non-precursor form. The hybrid DNA cloning techniques described as a route to the cloning and expression of HGH coding sequences in *E. coli* are generally applicable to other polypeptides which are synthesised initially as inactive precursors and later processed, or for which full length cDNA transcripts are unavailable.

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letters

On the isotropy and homogeneity of the Universe

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It is generally believed that the Universe began from a hot big bang. Among the clues to its very early history are the present day observations that the Universe is isotropic and homogeneous (as shown by the 3 K microwave background¹ and galaxy counts^{2,3}) and that there are about $10^{9\pm1}$ photons per baryon⁴. Penrose and others^{5,6} argue that these two observations imply that the Universe was initially very nearly isotropic and homogeneous. Their arguments rely on absolute baryon conservation so that the baryon number of the Universe can be used as a fiducial to which the number of photons can be compared. In recently proposed grand unified theories of particle interactions, baryon number is not absolutely conserved^{7,8} and thus the baryon number of the Universe is no longer a constant. It is argued here that in the absence of this fiducial the photon/baryon ratio is not a 'footprint' of the initial conditions, and therefore the initial singularity could well have been highly irregular as Misner suggested⁹.

The photon/baryon ratio is also $\approx$ the entropy/baryon ratio (more precisely, the specific entropy/baryon ratio) as nearly all the photons in the Universe are in the 3 K background. Misner suggested that the seemingly large entropy/baryon and the remarkable isotropy and homogeneity are related. His idea is that the initial singularity, rather than being isotropic and homogeneous, was highly irregular (chaotic), and the matter was cold (small entropy per baryon). Then, dissipative processes (pair creation, neutrino viscosity, and so on) smoothed out the irregularities and in the process also generated the large entropy/baryon. This is an attractive idea for several reasons. The Universe begins from a very general initial state (rather than isotropic and homogeneous), evolves into the observed state of isotropy and homogeneity, and thereby generates the observed entropy/baryon. However, calculations by Barrow and Matzner⁵ and arguments by Penrose⁶ indicate that in the process of dissipating irregularities, an initially highly chaotic geometry would generate more like 10^{40} photons/baryon. Therefore, they conclude that the observed entropy/baryon of $10^{9\pm1}$ implies that the initial geometry was very nearly isotropic and homogeneous.

This reasoning relied on comparing the entropy (number of photons) to an unchanging, non-zero number (in this case, the number of baryons in the Universe). However, recent theories in particle physics suggest that baryon number is not an exactly conserved quantity. The grand unification of the strong, weak and electromagnetic forces by a gauge theory seems to require that baryon and lepton conservation be abandoned^{7,8}. Unification puts quarks and leptons in the same multiplets, and therefore interactions exist which transform them into one another. In these theories baryon violating interactions are mediated by a super heavy boson (mass $m_x \geq 10^{14}$ GeV) and are almost negligible at low energies (the proton is nearly stable). However, these interactions would be of roughly the same strength as all the other interactions at energies above $\sim m_x (\geq 10^{14}$ GeV, temperatures above $\sim 10^{27}$ K). Several

authors¹⁰⁻¹⁷ have shown that these baryon non-conserving interactions along with CP violation ($K^0-\bar{K}^0$ system is known to violate CP) allow an initially hot ($T = 10^{32}$ K, the Planck temperature), baryon symmetric (net baryon number zero), isotropic and homogeneous Universe to evolve a net baryon excess when kT is $\sim m_x$. For reasonable values of the particle physics parameters the excess/entropy can be $\sim 10^{-9\pm1}$, and thus when the baryons and antibaryons annihilate, the Universe is left with $10^{-9\pm1}$ baryons per photon as we observe today. In addition, these grand unification theories predict a finite lifetime for the proton of $10^{31\pm1}$ years¹⁸ which may soon be accessible to measurement. The current lower limit on the lifetime of the proton is 10^{29} yr (ref. 19).

If these ideas are correct there may no longer be a conserved, non-zero quantity to which the entropy can be compared, and therefore the entropy cannot be used as a probe of the initial geometry of the Universe. For example, the Universe could have begun from a state with highly chaotic geometry and a definite number of cold baryons; very early ($t \sim t_{\text{planck}} \sim 10^{-43}$ s, $T \sim 10^{32}$ K), dissipative processes smoothed out the irregularities leaving the Universe isotropic and homogeneous, and with an entropy/baryon of $\sim 10^{40}$. When the temperature of the Universe dropped to about 10^{27} K ($kT \sim 10^{14}$ GeV) the baryon number and CP violating processes allowed the Universe to evolve a baryon/photon ratio of $\sim 10^{-9\pm1}$ erasing the only relic (baryon/entropy ratio $\sim 10^{-40}$) of a highly chaotic initial state.

One key assumption was tacitly made in the argument above: dissipation occurs before baryon generation. This assumption is needed because the baryon generation routes suggested thus far assume that the Universe is isotropic and homogeneous during the epoch of baryon creation ($kT \sim m_x$). In addition, any dissipation which occurs after the baryon excess is generated cannot be masked, as baryon number is very nearly conserved from that point forward. Thus we require dissipation to occur at a temperature $\geq m_x/k (\geq 10^{27}$ K). It has been pointed out that dissipative processes during the quantum gravity epoch ($t \sim 10^{-43}$ s, $T \sim 10^{32}$ K) would have been highly efficient in dissipating chaos^{5,6}, so that it is reasonable to believe that the Universe was isotropic and homogeneous for temperatures $\leq 10^{32}$ K. Barrow is also considering the case when the dissipation does not precede the baryon generation (personal communication).

If the dissipation occurs early enough, a new, non-zero conserved quantity is needed to gain knowledge of the initial geometry. In some of the candidate theories for grand unification in addition to charge, some other number may be absolutely conserved, usually $B-L$ (B is baryon number, L is total lepton number). Suppose that the net charge of the Universe is zero, that the charged particles of the Universe are protons and electrons, and that the leptons of the Universe are electrons, and the neutrinos and anti-neutrinos in the 2 K neutrino background. Assuming further that the number of neutrinos and anti-neutrinos are exactly equal, the lepton number of the universe is equal to the number of electrons. Since the number of neutrons is about 1/7 the number of protons, the net $B-L$ number of the Universe is, within an order of magnitude, equal to the baryon number of the Universe. Then once again there is a conserved, non-zero quantity to compare to the entropy and it is possible to use the observed (net $B-L$)/photon ratio (of $\sim 10^{-9}$) to infer that the Universe began from a nearly isotropic and homogeneous initial state.

However, it is not easy to measure the lepton number of the Universe since the 2 K neutrino background is not detectable

with present technology. Starting with the same assumptions as above except for the ratio of neutrinos to anti-neutrinos in the 2 K background which this time differs from 1 by $\sim 10^{-9}$; then for every $\sim 10^9$ photons there is one more neutrino than anti-neutrino. In this case the neutrino background contributes to the (net $B-L$)/photon $\sim -10^{-9}$, thereby reducing the (net $B-L$)/photon to nearly zero (say to 10^{-40}), whereas the visible (electrons, protons and neutrons) (net $B-L$)/photon is still $\sim 10^{-9}$. Therefore, while in principle the conserved quantity $B-L$ could be used to infer whether or not the initial geometry was isotropic and homogeneous, in practice, our inability to detect 2 K neutrinos makes this impossible.

The present day isotropy and homogeneity, and baryon/photon ratio of the Universe are important clues to the early history of the Universe. If a non-zero conserved quantity exists (such as baryon number), these two observations, as Penrose and others have pointed out, seem to imply the initial geometry of the Universe was nearly isotropic and homogeneous. However, if the grand unification ideas are correct and the baryon/photon ratio of $10^{-9 \pm 1}$ is generated in this way, then observed baryon/photon ratio, and the present isotropy and homogeneity do not allow us to infer that the initial geometry of the Universe was very nearly isotropic and homogeneous. In fact, the initial geometry could well have been highly chaotic.

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Quasar image doubling associated with absorption systems

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Ever since their discovery the origin of quasar absorption lines has been a subject of considerable controversy (see ref. 1). In this note we consider the consequences of the hypothesis that at least some of the absorption lines arise in galaxies. These galaxies could be ordinary ones with 50-100 kpc haloes², or perhaps more compact but more common (at $z \sim 1-2$) objects of comparable mass.

In addition to selectively absorbing light of certain wavelengths, galaxies bend the path of the light by gravitational attraction. In the weak field approximation, the amount of bending in passing a point mass M at a distance l_p is $4M/l_p$ in

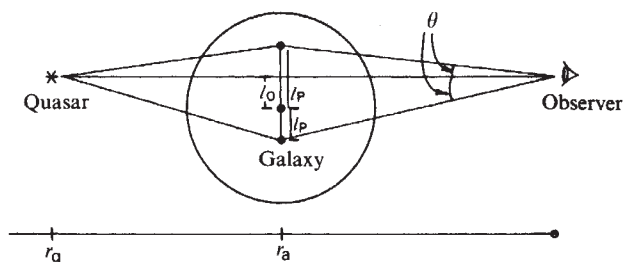


Fig. 1 The ray geometry is obviously not to scale. For precise definitions of the labels see text.

units $G = c = 1$ (ref. 3); if the mass is distributed and the light ray passes within it, the deflection, for the same total mass, can be slightly different depending on the details of the distribution^{9,10}. The order of magnitude of the deflection angle in passing through a galaxy is $\sim 0.6''(\Delta v/250 \text{ km s}^{-1})^2$, where $\Delta v = (GM/l_p)^{1/2}$ is the typical orbital speed in the galaxy. Such a small amount is invisible to ground-based optical telescopes (though not by a large factor), but should be easily discernible by either the Very Large Array (VLA) or the Space Telescope. We suggest, therefore, that those quasars with well defined absorption systems should be searched for gravitationally-focused multiple images.

More precisely, we suppose that an absorption system of redshift z_a implies the presence of a galaxy at that redshift. We may find the angle by which the ray reaching Earth differs from the undeflected line of sight past the galaxy from the expression⁴:

$$4\left(\frac{\Delta v}{c}\right)^2 f(l_p) \frac{r_a r_{aq}}{(1+z_a)r_q} = l_p \mp l_o = \frac{r_a \theta}{1+z_a} \quad (1)$$

where f is a slowly varying function of order unity whose form depends on the shape of the galaxy mass distribution; r_a and r_q are the comoving radial coordinates of the galaxy and the quasar, respectively, with respect to the observer as origin, whereas r_{aq} is the comoving radial coordinate of the galaxy with respect to the quasar as origin; l_o is the proper distance from the centre of the galaxy to the undeflected line of sight and l_p is the proper pericentre distance of the ray; and θ ($\ll 1$) is the angle between the undeflected line of sight and the received ray (see Fig. 1). Here we have made the approximation that $l_p \approx \pm l_o + (r_a \theta)/(1+z_a)$; it is actually slightly less. The $\pm$ correspond to paths that lie on the same, or opposite, sides of the galaxy's centre as the undeflected line of sight. We have also assumed that there is no other scattering along the ray path. In cosmologies which are homogeneous only in the mean so that the rays propagate in empty space but are subjected to many weak scatterings^{5,6}, the results differ by $<10\%$.

The function $f(l_p)$ accounts for the galaxy's mass distribution. If it is spherically symmetric and has a flat velocity curve from inside l_p out to a radius l_1 (but has no mass outside l_1), then we have (for $l_p < l_1$):

$$f(l_p) = \frac{1}{2}(\cos^{-1}(l_p/l_1) + 2(l_1/l_p)\{1 - [1 - (l_p/l_1)^2]^{1/2}\}) \\ \approx \begin{cases} \frac{1}{2}[\pi + \frac{1}{6}(l_p/l_1)^3] & l_p/l_1 \ll 1 \\ 1 & (l_1 - l_p)/l_1 \ll 1 \end{cases} \quad (2)$$

If $l_p \geq l_1$, $f(l_p) = l_1/l_p$.

The easiest way to find the actual splitting angle (given l_o , Δv , and the cosmological information) is to use the graphical construction of Fig. 2. The splitting angle is:

$$\theta_{sep} = 4[f(l_{p+}) + f(l_{p-})]\left(\frac{\Delta v}{c}\right)^2 \frac{r_{aq}}{r_q} \\ \approx 0.9'' r_{aq}/r_q \left(\frac{\Delta v}{250 \text{ km s}^{-1}}\right)^2 \quad (3)$$

where $l_{p\pm}$ are given by the two intersections of the straight lines with the curve in Fig. 2.

Clearly, there are only solutions for both l_{p+} and l_{p-} if:

$$l_0 \leq \pi \left(\frac{\Delta v}{c} \right)^2 \frac{r_a r_{aq}}{(1+z_a)r_q} = 13 \frac{r_a r_{aq} H_0}{(1+z_a)r_q c} \left(\frac{\Delta v}{250 \text{ km s}^{-1}} \right)^2 \times \left(\frac{H_0}{50 \text{ km s}^{-1} \text{ Mpc}^{-1}} \right)^{-1} \text{ kpc} \quad (4)$$

To evaluate this limit, we note that³:

$$r_a = \frac{c}{H_0(1+z_a)q_0} \{z_a q_0 + (q_0 - 1)[-1 + (1 + 2q_0 z_a)^{1/2}]\} \quad (5)$$

with an analogous expression obtaining for r_q , while

$$r_{aq} = \frac{c}{H_0 q_0^2 (1+z_a)(1+z_q)} [(1 - q_0 + q_0 z_q)(1 + 2q_0 z_a)^{1/2} - (1 - q_0 + q_0 z_a)(1 + 2q_0 z_q)^{1/2}] \quad (6)$$

where z_q is the quasar redshift and q_0 , H_0 are evaluated at the present epoch. For 'typical' numbers ($q_0 \leq 0.1$, $0.5 \leq z_a$, $z_q \leq 3.5$), the maximum undeflected impact parameter that will produce a double image is only

$$\sim 2 \left(\frac{\Delta v}{250 \text{ km s}^{-1}} \right)^2 \left(\frac{H_0}{50 \text{ km s}^{-1} \text{ Mpc}^{-1}} \right)^{-1} \text{ kpc}$$

(see Table 1).

Consequently, only a small fraction, $\langle (l_{0, \text{max}}/l_1)^2 \rangle$, of all absorption systems will produce double images. To produce the six or so absorption systems which are commonly found in quasars of $z_q \approx 2$,

$$l_1 \approx 100 \text{ kpc} \left(\frac{H_0}{50 \text{ km s}^{-1} \text{ Mpc}^{-1}} \right)^{-1}$$

(ref. 7). This estimate of l_1 assumes the present number density of galaxies and no galaxy-quasar correlations; it would decrease if the density of objects gravitationally bound as tightly as galaxies and at least a few kiloparsecs in size were greater at $z \sim 1-3$, or if correlations do exist. Using $l_1 \approx 100 \text{ kpc}$, we

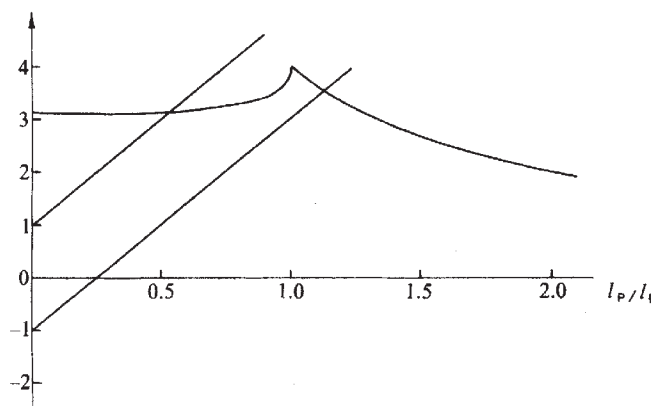


Fig. 2 The curve shows $4f(l_p)$ as a function of l_p/l_1 for the mass model that led to equation (2). The lines represent $(l_p \pm l_0) \cdot (1+z_a)r_q/[r_a r_{aq}(\Delta v/c)^2]$ as a function of l_p/l_1 , where we have chosen the illustrative parameters:

$$\frac{r_a r_{aq}}{(1+z_a)r_q} \left(\frac{\Delta v}{250 \text{ km s}^{-1}} \right)^2 \left(\frac{l_1}{100 \text{ kpc}} \right)^{-1} \left(\frac{H_0}{50 \text{ km s}^{-1} \text{ Mpc}^{-1}} \right)^{-1} = 6$$

and $l_0/l_1 = 0.25$.

Their intersections with the curve give the approximate pericentres of the received rays: $l_p/l_1 \approx 0.53$ and 1.13 . As l_0/l_1 increases, the intercepts of the straight lines with the vertical axis spread until eventually only the ray corresponding to the - sign is received.

Table 1 Sample values of θ_{sep} and the maximum ray impact parameter $l_{0, \text{max}}$

z_a	r_a	r_{aq}	$l_{0, \text{max}}(\text{kpc})$	$\theta_{\text{sep}}(\text{arc s})$
0.25	0.22	1.04	1.76	0.69
0.5	0.41	0.81	2.10	0.54
1.0	0.71	0.50	1.70	0.33
1.5	0.95	0.29	1.06	0.19
2.0	1.17	0.13	0.49	0.086

$q_0 = 0.1$ and $z_q = 2.5$ ($r_q = 1.364c/H_0$)

The values of r_a and r_{aq} are in units of c/H_0 ; $l_{0, \text{max}}$ and θ_{sep} are calculated assuming $(\Delta v/250 \text{ km s}^{-1})^2 \times (H_0/50 \text{ km s}^{-1} \text{ Mpc}^{-1})^{-1} = 1$.

estimate the probability per absorption system that a double image is formed to be $O(10^{-3})$. Unfortunately, the close approach of the rays to the galaxy centre also entails the hazard that visible wavelengths may be extinguished by dust, or that the surface brightness of the galaxy may mask the quasar images.

Another way of understanding this probability estimate is to use the result⁵ that if the mass of the Universe comes in many point-like objects, the probability of creating a visible double image of an object at a cosmological distance is of order Ω , the density parameter. Here, in effect, only the mass contained in the inner few kiloparsecs contributes to Ω .

There are two types of checks that may be made on a double image, to confirm that it is due to gravitational deflection. The first, which must await the Space Telescope, is to match the spectra of the two images. In general, radio spectra are too smooth to attach much significance to matching their shapes. The second is to match the images' time-variability records. The difference in path lengths will be

$$\approx 2 \left(\frac{\Delta v}{250 \text{ km s}^{-1} \text{ Mpc}^{-1}} \right)^4 \left(\frac{H_0}{50 \text{ km s}^{-1} \text{ Mpc}^{-1}} \right)^{-1} (r_{aq}/r_q)^2 \text{ months},$$

depending on how nearly symmetrical the ray paths are about the galaxy centre. Even if the two images are not resolved, the quasar can be examined for doubling by performing an autocorrelation in time of its intensity (S. Tremaine, personal communication).

The autocorrelation function will always have its strongest peak at zero delay, but if the intensities of the images are not too different, a second peak should appear that corresponds with the difference in path lengths. To estimate that intensity ratio, we use the fact that the gravitational lens conserves surface brightness. Then each image's intensity is multiplied by a factor $l_p/l_0 dl_p/dl_0$ (ref. 8); in our case, $d \ln f(l_p)/d \ln l_0 \ll 1$, so the factor reduces to $\approx l_p/l_0$. Thus we expect an intensity ratio of perhaps a factor of two or so, and the autocorrelation test should be feasible.

The plausible idea that at least some quasar absorption systems are made in galaxies implies that a small fraction of these galaxies will cause a doubling of the quasar image by gravitational focusing. The separation of these images ($\leq 1''$) should be detectable with the VLA (for radio quasars) and the Space Telescope when it flies. Autocorrelation of the variability of unresolved quasar images may also reveal doubling due to gravitational focusing.

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Scaling law test and two predictions of planetary magnetic moments

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The scaling law for the strength of planetary magnetic dynamos, originally derived from a magnetohydrodynamic (MHD) theory¹, has recently been independently re-derived using simple dimensional analysis². While dimensional analysis is certainly no replacement for a solution based on a physical mechanism, this analysis does lend more credence to the scaling law by the implicit suggestion that the form of the scaling law is not an artefact of the MHD solution. We show here that the scaling law holds quite well in ordering the three well determined planetary magnetic moments. The present meagre data suggest that the terrestrial magnetic moment is about twice the value expected when compared with the other planets. Assuming that Earth is anomalous, rather than Mercury and Jupiter, and that the scaling law is valid, we predict that Saturn will be found to have a moment of $1.2 \times 10^{29} \text{ G cm}^3$ and Io a moment of $6.5 \times 10^{22} \text{ G cm}^3$.

Expressed in terms of magnetic moments which are the observable parameters in planetary exploration, the scaling law becomes

$$\frac{M_P}{M_E} = \left(\frac{\rho_P}{\rho_E} \right)^{1/2} \left(\frac{R_P^c}{R_E^c} \right)^4 \frac{\Omega_P}{\Omega_E}$$

where M is the magnetic moment, R^c is the radius of the core, ρ is the density of the core and Ω is the spin rate. The subscript P refers to the planet, E to the Earth. For this scaling law to work, all planetary dynamos must have the same underlying physical mechanism. We note also that the strength of the energy source for the dynamo does not appear in this scaling law. In this model if the strength of the energy source is not sufficient, the dynamo does not regenerate and then stops. If it is regenerative, it assumes a particular strength given by the scaling law. This lack of consideration of the strength of the energy source is considered by many to be grounds for rejection of any scaling law.

In the scaling law Ω is well known for all large bodies in the Solar System except for the outermost planets. However, there is much uncertainty in the core radius and density, especially for the inner planets whose chemical composition and thermal history are uncertain. Nevertheless, enough modelling has been done, and planetary measurements performed for us to be able to put this scaling law to a serious test.

The results of this test are shown in Fig. 1. For Mercury we have made the core density the same as that of the Earth and the limits on core radius to be 1,470–1,840 km (refs 3, 4). The range of the observed magnetic moment was taken to be from 2.4×10^{22} to $5 \times 10^{22} \text{ cm}^3$ (refs 5–7). For Venus we have taken a core density equal to that of the Earth and a fractional radius within 10% of that of the Earth. The magnetic moment has been taken to be less than $1 \times 10^{22} \text{ G cm}^3$ (ref. 8). For the Earth we have taken a moment of $8 \times 10^{25} \text{ G cm}^3$, a radius of 3,486 km, and a density of 11 g cm^{-3} (ref. 9). For Mars, we have taken a range of models with realistic core densities given by Okal and Anderson¹⁰ for the internal structure and the magnetic moment of Dolginov and coworkers¹¹ as an upper limit¹². For Jupiter we use the models of Stevenson and Salpeter and its stated uncertainties¹³ and the moment of Davis and Smith¹⁴.

The straight line through the Earth has a slope of unity. We would expect that those planets with operating dynamos would fall on this line. Clearly Jupiter and Mercury do not. However, they both fall on a slightly displaced line with unit slope. Thus relative to Jupiter and Mercury the Earth seems to be

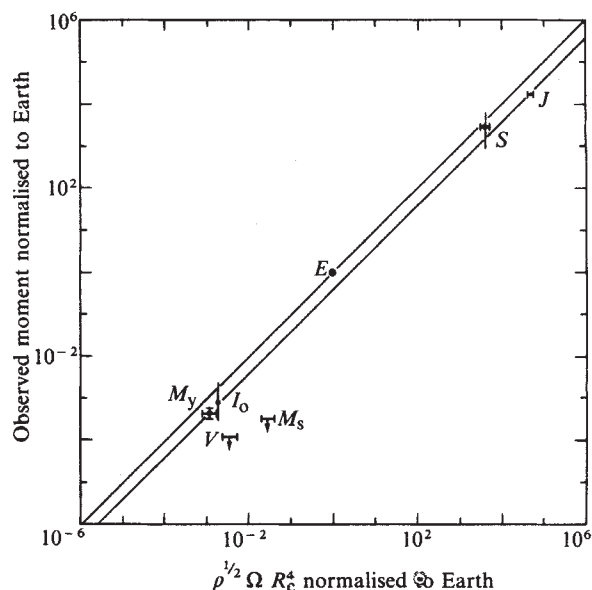


Fig. 1 Application of the scaling law to Earth (E); Mars (M_s); venus (V); Mercury (M_y); Saturn (S); Jupiter (J) and Io.

anomalously high. It might be said that since the three planets with clearly established dynamos do not fall on one line, then the scaling law has failed. However, for the sake of further discussion, we will assume that the scaling law works but that the Earth is anomalous.

Venus and Mars do not lie near either line which suggests that they do not have dynamos at present. If one were to detect unambiguously an intrinsic field at Venus or Mars due to an internal dynamo at or below these limits, this observation would necessitate reassessment of Busse's dynamo theory.

For Saturn we have taken Slattery's model¹⁵ and arbitrarily assumed $\pm 6\%$ for the uncertainty in core radius. The dot marks the moment inferred from radio observations¹⁶. This data point falls squarely between the jovian and terrestrial scaling lines. If we assume that the jovian scaling line is the proper scaling line with which to compare Saturn we would predict a core radius for Saturn of 33,000 km, much greater than the 28,000 km assumed by Slattery. If the modelling is correct, then the expected moment is $1.2 \times 10^{29} \text{ G cm}^3$ corresponding to an equatorial surface field of 0.5 G. While it is difficult to judge which is the more accurate, the theoretical modelling of Saturn's interior or the scaling of the radioemissions, we argue that as the source of the radioemissions is not understood for the Earth, Jupiter or Saturn, the modelling effort is more accurate at present. Thus, our first prediction is that Pioneer 11 will measure a moment of about $1.2 \times 10^{29} \text{ G cm}^3$. If the jovian modelling efforts can be of any guide we expect this estimate to be correct to about $\pm 20\%$.

Finally let us examine Io. A prediction for Io has already been made by Neubauer¹⁷. He assumes an Fe-FeS eutectic core with a density of 5.3 g cm^{-3} and a core radius of 890 km corresponding to an average chondritic composition. Voyager pictures of Io show many active volcanoes suggesting that Io is molten internally. This in turn suggests Io has in fact differentiated and formed an iron core. Thus far the Voyager mission has not provided further constraints on the size of the core. However, it is clear that the satellite is losing sulphur. Thus we will also use a higher core density, 7 g cm^{-3} (ref. 10) in our estimates of the expected moment. This expectation is indicated by the vertical line which crosses the Earth scaling law at a moment of $1.3 \times 10^{23} \text{ G cm}^3$, very close to Neubauer's prediction. The line crosses the Jupiter-Mercury line at $6.5 \times 10^{22} \text{ G cm}^3$. This same moment has been inferred from observations of Io's ionosphere and effects on particle motions by Kivelson and coworkers¹⁸. Our second prediction, based on the adoption of the Jupiter-Mercury line as the best scaling line, is that Io has a moment of

close to $6.5 \times 10^{22} \text{ G cm}^3$. The weak link in this prediction is the choice for the size of the core of Io.

If the above predictions are correct, we must seek an explanation for why the Earth's field is anomalously high, as the scaling law does not involve the strength of the energy source. Perhaps the answer lies in the precessional torques applied to the Earth's core by the Moon.

I thank D. J. Stevenson and S. K. Runcorn for helpful discussions.

Note added in proof: On 1 September 1979, Pioneer 11 flew by Saturn and detected a magnetic moment of $4.8 \times 10^{28} \text{ G cm}^3$ (E. J. Smith, personal communication). To be consistent with the Busse scaling law, the conducting core of Saturn would have to be 23,000 km in radius. Unless future analysis of the Pioneer 11 gravity data and a reassessment of the models of the interior structure are consistent with such a small core we must conclude that Busse's scaling law is useful for predicting planetary moments only to within a factor of about 2.5.

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Isotope effects and correlated motion in self and sodium ion diffusion in water

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The information gained on atomic motions in solids from studies of the isotope effect in diffusion^{1,2} prompted us to examine this effect in liquids, in particular for diffusion in water, because of its importance as a solvent. As isotope effect measurements for self-diffusion in water and deuterium oxide are available³, the isotope effect for ²²Na and ²⁴Na ions in water was studied. We describe here a new technique which applies to liquids the solid state technique of simultaneous diffusion⁴ of these isotopes followed by sectioning and half-life (or other suitable method) separation.

A dilute sodium chloride solution (0.034 mol l⁻¹) was gelled by the addition of between 1 and 3% agar (Ion Agar No. 2) and cast into a tube from which it could be pushed by an accurate screw thread and plunger for sectioning into slices 1–3 mm thick. Suitable quantities of ²²NaCl (carrier free ²²Na, Radiochemical Centre, Amersham) and ²⁴NaCl (prepared by irradiation of NaCl in the Scottish Universities Research Reactor) in aqueous solution were dripped onto a freshly cut gel surface from a hypodermic syringe before the tube was capped for annealing in a temperature controlled bath.

After sectioning, the gel slices were weighed and centrifuged down into suitable counting tubes to provide a reproducible counting geometry and each counted repeatedly in a well-type NaI scintillation counter until the 15-h half life ²⁴Na had

completely decayed. From the decay curve for each section the specific activities C_{22} and C_{24} for each tracer could be calculated.

Plots of the log of the specific activity against the square of the penetration distance were accurately linear over a two decade range in activity, as required by the appropriate solution of Fick's equation. Moreover, comparison of the resulting diffusion coefficients with those obtained by conventional methods showed that the sodium diffusion was taking place through the water phase.

The isotope effect was then calculated from the equation¹⁶

$$\ln (C_{22}/C_{24}) = \text{constant} - (D_{22}/D_{24} - 1) \ln (C_{22})$$

The results obtained for the isotope effect for sodium diffusion in water are listed in Table 1 together with the self-diffusion results of Mills³ and one value for ⁶Li and ⁷Li calculated from the limiting conductance data of Kunze and Fuoss⁵.

The results were analysed using Schoen's equation⁶

$$\left(\frac{D_a}{D_b} - 1\right) = f \Delta K \left[\left(\frac{m_b + nm_0}{m_a + nm_0}\right)^{1/2} - 1 \right] \quad (1)$$

where D_a/D_b is the ratio of the diffusion coefficients of the two isotopes in the same solvent, f is the correlation factor for the diffusion process, m_a and m_b are the masses of the isotopic tracers and n and m_0 the number and average mass of any non-tracer molecules involved in the diffusion movement. In solids ΔK is the fraction, possessed by the diffusing species, of the total translational kinetic energy associated with the decomposition of the saddle point configuration¹. Clearly $0 \leq \Delta K \leq 1$ and in many metals, particularly the close packed metals², ΔK is close to unity.

Although Schoen's equation is exact only in certain conditions^{1,7}, it has been applied to glass⁸ and benzene⁹ where the required symmetry conditions would not be met. Water has a structure which, at least over short distances, approximates to a tetrahedral arrangement of water molecules^{10–13} with Z , the coordination number, lying between 4.4 and 5.5. For such a molecular arrangement equation (1) should hold at least to the precision justified by the experimental data.

Table 1 shows values of $f\Delta K$ calculated from equation (1). For water $n = 0$, while for sodium and lithium m_0 is the mass of a water molecule and a value of $n = 4.5$ was used in view of the known hydration of alkali ions in water. If the hydration number is defined as the number of water molecules which have surrendered their own translational freedom and remain with the ion when it moves relative to the surrounding water, then recent reviews^{14,15} suggest that the most probable integral value for n is 4 ± 1 for sodium and 5 ± 1 for lithium. The value of $n = 4.5$ chosen is then a very convenient test value for both ions; it is probably close to the best possible estimate for sodium¹⁵ but may be slightly low for lithium.

It is encouraging that the values of $f\Delta K$ so calculated for water and the hydrated alkali ions are so similar. This is particularly true for the alkali ions since in that case the experimental techniques used were so different.

It has been shown elsewhere^{1,16} that the correlation factor f_0 for self-diffusion by a vacancy or hole mechanism can be approximated with a fair degree of accuracy by

$$f_0 = 1 - \frac{2}{Z} \quad (2)$$

where Z is the coordination number. This is very important when applying the idea of correlated motion to liquids since long-range order can be neglected and attention focussed on the number of neighbours in the first coordination shell.

The striking agreement between the experimental $f\Delta K$ for water and the range of values of f_0 calculated from equation (2) supports a hole mechanism for diffusion in water and suggests that ΔK is close to unity, $f_0 \approx 0.62$ and Z is about 5 (ref. 12). Note that a ΔK of 1 implies that the frequency with which a tracer moves into a hole is inversely proportional to the square root of its mass and this is in line with theoretical predictions¹⁷.

Note that molecular motion in water may be strongly correlated. Indeed, as

$$D(\text{measured}) = D(\text{random}) f_0$$

the observed diffusion coefficient may be about 40% less than would be calculated on the basis of random motion. This accords with predictions made on the basis of molecular dynamics¹⁸. This interpretation differs from that of Dunlop¹⁹ who suggested that $f_0 = 1$ and $\Delta K \approx 0.5$.

For sodium and lithium also ΔK can be assumed to be close to one. For sodium f would be expected to exceed that for water diffusion as it is a slow diffusing impurity.

The results shown in Table 1 can be interpreted on the basis of a crude model. Suppose each alkali ion is surrounded symmetrically by between 4 and 6 hydrated water molecules²⁰, the whole loosely contained in a cage of free water. On average the hydrated water will have fewer free molecules adjacent to it than a free molecule because of the presence of the sodium ion and the remainder of its hydration cell on one side. If a complete hemisphere were forbidden then each hydrated water molecule would have on average 2.5 free molecules adjacent to it. If we assume an integer value of 3 for the average then each hydrated water with its three free water molecules and the alkali ion forms a structure having a coordination number of four.

Suppose that a space occurs in the shell of free water adjacent to a hydrated water molecule. That hydrated molecule can then move into the space carrying with it the hydrated ion. This produces a space next to the free molecules on the opposite side of the hydrated ion. One of these free molecules could move to occupy the space or alternatively the hydrated alkali ion could move back to occupy its original position.

This motion of hydrated alkali ions and free molecules closely resembles an impurity molecule diffusing on a diamond lattice for which an expression for f is available¹. If the relative probabilities of a hydrated or free water molecule moving into a space are described in terms of jump frequencies ω and ω_0 then the correlation factor for hydrated alkali ion motion in such a structure is

$$f = \frac{\omega_0}{\omega + \omega_0} \quad (3)$$

which for the case of self-diffusion where $\omega = \omega_0$, reduces to $f = 0.5$ as required by equation (2). If $\omega_0 > \omega$ then $f > 0.5$.

If we assume that the probability of a hole forming in the shell adjacent to a hydrated ion is the same as in pure water then, as the mean distance moved in each step is the same for free or

Table 1 Isotope effects and correlation factors for self and impurity diffusion in water

System	Temperature (°C)	$(D_a/D_b - 1)$	$f\Delta K$	f_0 (Theory)
HDO/HTO in H ₂ O	1	0.0135	0.52	
	5	0.0181	0.69	0.55
	25	0.0161	0.62	to
	45	0.0167	0.64	0.64
	Average	0.0161	0.62	
HDO/DTO in D ₂ O	5	0.0330	0.64	
	15	0.0287	0.56	0.55
	25	0.0299	0.58	to
	Average	0.0305	0.59	0.64
²² Na/ ²⁴ Na in H ₂ O	19	0.0059		
	19	0.0065		
	19	0.0056		
	30	0.0066	0.63 ± 0.03	
	39	0.0057		
	50	0.0062		
	Average	0.0061		
⁶ Li/ ⁷ Li in H ₂ O	25	0.0035	0.61	

Table 2 Correlation factors and hydration numbers for alkali diffusion in water at 25°C

System	Observed ratio	Calculated ratio	f	n	ω_0/ω
$D(\text{H}_2\text{O})$ $D(\text{Na})$	$\frac{2.236}{1.296} = 1.72$	1.68	0.64	4.6	1.8
$D(\text{H}_2\text{O})$ $D(\text{Li})$	$\frac{2.236}{1.030} = 2.17$	1.59	0.71	5.3	2.5

The units of D are $10^{-5} \text{ cm}^2 \text{ s}^{-1}$. The values of the tracer diffusion coefficients of water and sodium are from refs 3 and 22. The value for lithium was calculated from the limiting conductance, ref. 23.

hydrated water, the ratio of the coefficients of self and impurity diffusion can be written as

$$\frac{D(\text{H}_2\text{O})}{D(\text{ion})} = \frac{\omega_0 f_0}{\omega f} \quad (4)$$

This assumption is plausible as both sodium and lithium are 'structure-forming' ions, known not to perturb the structure of water. In the case of lithium, molecular dynamical calculations also suggest that the Li⁺ ion causes relatively little disturbance of the structure of the surrounding water²⁰. Note that this assumption cannot be extended to other ions. For example, the addition of caesium ions to water enhances water self-diffusion²¹, probably because of a greater ease of formation of holes adjacent to the caesium ion.

Eliminating ω_0/ω from equations (3) and (4) yields the simple relationships

$$\frac{D(\text{H}_2\text{O})}{D(\text{ion})} = \frac{f_0}{1-f} \quad (5)$$

and

$$\frac{\omega_0}{\omega} = \frac{f}{1-f} \quad (6)$$

for this two frequency model.

Table 2 gives experimental values for the left-hand side of equation (5) at 25°C for sodium and lithium together with calculated values using the f and f_0 values from Table 1. The agreement is surprisingly good, particularly for sodium, considering the crudeness of the model and the assumptions made.

An alternative application of equation (5) is to use the experimental values of $D(\text{H}_2\text{O})/D(\text{ion})$ and f_0 to calculate f and then, using the experimental values for the isotope effect and Schoen's equation, (1), to calculate independently values of the hydration number. The resulting values of f , and n are shown in Table 2.

The values of n so obtained are in excellent agreement with those obtained by other methods and support the conclusion that the hydration number for lithium slightly exceeds that for sodium.

Finally, values of ω_0/ω calculated using the f values from Table 2 are also listed. In the case of lithium this ratio is almost equal to the inverse square root of the masses of the hydrated ion and water.

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Solar bacterial biomass bypasses efficiency limits of photosynthesis

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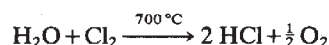
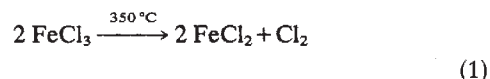
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A new kind of solar energy technology is proposed here which will allow biomass to be produced at efficiencies higher than the theoretical limits of photosynthesis and at one order of magnitude higher than by present processes. Its principle is to produce the chemically simple inorganic energy source of certain autotrophic bacteria by solar thermal, photoelectrochemical or solar powered electrochemical methods and to grow these bacteria, for example *Thiobacillus ferrooxidans*, which can live from the oxidation of ferrous to ferric iron in continuous cultures in the dark. A remarkable prospect of this novel strategy for gathering solar energy would be that biomass for food, fuels and materials could be produced in largely automatic and compact factory-type installations on arid and infertile land, to which the limited amount of water which is needed to fix CO₂ and is extracted in the form of dry organic products may be transported as a raw material.

The efficiency limits of photosynthesis are well known. Insufficient photon energy and incomplete utilisation of photon energy reduce the upper theoretical limits of the conversion efficiency to approximately 11% of the total solar radiation on Earth. Inevitable nongenerative losses and plant system losses (such as reflection, and metabolic conversions) further reduce the maximum practical conversion efficiency to 5–6%¹. High daily yields approaching this maximum have been reported, but even very productive tropical grasses such as sugar cane do not yield crops at efficiencies of more than 0.6% on an annual basis². The water hyacinth, which is considered to be one of the most efficient solar energy converters is expected to be capable of yields of 45–60 tonnes acre⁻¹, corresponding to ~3–4% efficiency under careful management¹. Unfortunately, the need for fertile soil and abundant sweet water restricts biomass production mostly to regions which to a large extent are already cultivated. It cannot be extended to the huge unused desert regions of the world where solar energy is at its most intense.

The rather low efficiency limits of biomass production and the high loss of water which is primarily due to evaporation from the plants and the soil can be overcome by the proposed biomass technology. This could replace the inefficient multi-photon biological mechanism of conversion of solar energy into energy-rich primary products by more efficient artificial solar techniques, which could provide either concentrated solar heat or solar electricity for the production of chemically simple inorganic energy carriers on which microorganisms feed.

I have cultivated and studied the bacterium *T. ferrooxidans* for several years³, and to demonstrate the feasibility of such a solar technology I have theoretically investigated a biomass installation, in which this bacterium is grown on ferrous iron produced from ferric iron by a solar thermal method. In analogy to the generation of hydrogen and oxygen from water by thermal processes many chemical cycles can be proposed which evolve oxygen from water and reduce ferric ions instead of protons. The estimated efficiencies of proposed thermal processes to generate H₂ and O₂ from water range from 30 to 50% (refs 4–7) and will not be lower for the production of ferrous iron, which has a considerably more positive redox potential than that of hydrogen. While no practicable thermochemical processes with less than three steps are known for the production of H₂ and O₂ from water, two-step processes are possible for the generation of ferrous iron and oxygen. An example are the reactions:



T. ferrooxidans requires an acid environment (*p*H = 1.2–4.5) and obtains its energy for growth from oxidising ferrous iron, metal sulphides, sulphur or soluble inorganic sulphur compounds. It grows in strictly autotrophic conditions (optimum at 35 °C), obtaining all the carbon required for biosynthesis from atmospheric carbon dioxide which it fixes by biochemical mechanisms that are part of or related to the Calvin cycle⁸. Ammonium is a suitable source of nitrogen, but evidence for the ability of *T. ferrooxidans* to fix atmospheric N₂ has been presented⁹. The bacterium develops a very high tolerance for metal ions. Cl⁻ retards Fe²⁺ oxidation at concentrations above 6 g l⁻¹, but tolerance to cultivation in hydrochloric acid can be developed and its growth on iron in a medium where all iron is supplied as the chloride is possible provided some additional sulphate is available¹⁰. *Thiobacillus ferrooxidans* can therefore be cultivated on FeCl₂ supplied by the solar thermal process (1) and thereby recycles FeCl₃ and water:



T. ferrooxidans accomplishes the oxidation of Fe²⁺ at rates 10⁵–10⁶ times faster than the rates of inorganic oxidation at the corresponding *p*H (ref. 8). The energy which it thereby gains depends on the *p*H and ranges from 7.84 kcal mol⁻¹ (*p*H 1.5) to 5.89 kcal mol⁻¹ (*p*H 3). For the reduction of CO₂ to the level of carbohydrate approximately 118 kcal mol⁻¹ CO₂ are needed. For an output of 7 kcal mol⁻¹ Fe²⁺ about 16 g mol⁻¹ Fe²⁺ would be consumed to fix 1 g mol⁻¹ CO₂. Per gram mol Fe²⁺ (56 g) a maximal theoretical cell yield of approximately 1.4 g dry weight could therefore be expected⁸. The energy conversion efficiencies actually obtained by *T. ferrooxidans* grown on ferrous iron have steadily improved with the development of improved strains and the use of continuous flow chemostat cultures. A detailed recent analysis of growth yields and total energy balance at *p*H 1.6 is given by Kelly *et al.*¹¹, which suggests that 19 Fe²⁺ ions are oxidised per CO₂ molecule fixed. The true growth yield Y_G (g dry weight per atom Fe²⁺, if no substrate were used for the maintenance of the bacteria) was found to be 1.33, corresponding to an energy conversion efficiency of 78.8%. The yield actually obtained, which includes the energy losses due to cellular processes and is subject to non-competitive and competitive product inhibition by the ferric iron, varied depending on the condition of cultivation between 0.156 and 0.8 g dry weight per g atom Fe²⁺ (ref. 11). A dry weight yield of 0.5 g per g atom Fe²⁺ corresponding to an efficiency of 35% on a free energy basis has also been reported^{8,12} and I believe that is a reasonably achievable value for large scale technical biomass production by *T. ferrooxidans* in continuous culture.

If this efficiency (*η*₂ = 35%) is combined with the efficiency for the solar thermal generation of ferrous iron (*η* = 30–50%) a total solar energy efficiency for biomass production of *η* =

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$\eta_1\eta_2 = 10.5\text{--}17.5\%$ is obtained for the proposed solar technology. On an annual basis it would thus be 17.5 to nearly 30 times higher than the biomass yield from a sugar cane field of comparable area. The bacterial biomass would be very protein-rich (*T. ferrooxidans* contains 44% protein, 26% lipid, 15% carbohydrate and at least two B-vitamins¹³), has no known negative physiological effect and could be collected in installations of moderate size, since *T. ferrooxidans* can be cultivated on high concentrations of ferrous iron, achieving growth rates exceeding 0.2 h^{-1} and doubling times $<3.5\text{ h}$ in continuous flow cultures¹⁴. Using published experimental data¹¹ biomass yields of $70\text{ kg dry wt m}^{-3}\text{ yr}^{-1}$ can already be estimated for continuous cultures in their present stage of development. Single cell protein factories have already been shown to be feasible in the case of petroleum conversion, and problems arising from the processing of the material for specialised use seem to be solvable.

What would a solar bacterial biomass (SBB) plant look like? Built as a solar tower installation with a collector field of 1 km radius in a desert it would cover an area of 3.14 km^2 . As not all the incident sunlight could be collected in the central receiver the input of solar energy would approximately correspond to that of a comparable sugar cane field in a tropical region with inferior insulation. However, up to 30 times more biomass could theoretically be extracted from the SBB installation which could be built on infertile soil. The thermochemical reactions (1) and the energy supplying reaction (2) on which bacterial biomass is grown recycle the water needed to cultivate *T. ferrooxidans* and the only real loss of water is due to the biological fixation of CO_2 . If biomass is extracted from the SBB plant as dry material the weight equivalent of water which would have to be replaced would be smaller or—if some losses are taken into account—of a similar order of magnitude. Provided that the water in the SBB installation is periodically cleaned, which could occur during the thermochemical step (1), all that may be needed is that those transportation vehicles which carry away dry biomass also supply water to the plant. The total amount of water needed to operate a SBB plant would fill tanks (10 m high) which cover $<10\%$ of the collector area (mirrors can be placed on top of these). The addition of a fertiliser to the culture medium will be necessary but the bacteria's apparent ability to utilise nitrogen from the air⁹ could be developed. (A standard culture medium has been described in ref. 15).

The described energy collecting cycle for a SBB installation based on *T. ferrooxidans* and a solar thermal process is just one technical possibility which is especially interesting since it promises high theoretical efficiencies. One disadvantage, which is common to all thermochemical cycles, is that relatively large quantities of material (Fe^{2+} , Fe^{3+}) would have to be circulated to maintain a relatively small flow of energy. The simplicity of the reactions involved (1)(2), and a skilful technical design (for example, utilisation of thermal convection) might keep such additional energy losses within reasonable limits. They would not occur if the solar thermal process for the generation of Fe^{2+} were replaced by a solar powered electrochemical one (current generation by solar cells, ocean thermal gradient, wind, wave power) or a photoelectrochemical one in which ferrous iron is produced in the cathodic reaction and oxygen in the anodic reaction of an electrochemical cell. In this case the bacteria could in principle be grown in the same reactor in which their energy source is produced and they would be converting irregularly supplied electrical energy into valuable organic products. The total efficiency ($\eta = \eta_1\eta_2$) which would seem to be feasible in this case is of the order of 6% . The same bacterium *T. ferrooxidans* could also be cultivated on sulphur, thiosulphate, many metal sulphides or a combination of metal sulphides with ferric iron provided the energy supplying inorganic substances can be efficiently recycled from the oxidation product sulphate by a solar thermal or solar electrochemical process. *Thiobacillus thiooxidans* could be grown on sulphur or inorganic sulphur compounds and *Knallgasbacteria* on solar hydrogen. There are other bacterial species which feed on energy sources which are

chemically sufficiently simple to be synthesised by solar technological methods.

The suggested SBB technology is based on established principles and does not involve expensive raw materials. It also seems that certain practical problems which will arise (for example, the separation of bacterial biomass from ferric iron) could be handled with appropriate applied research. These preconditions, the promise of biomass yields, which are one order of magnitude higher than that from present day agriculture, extreme economy of water utilisation and the prospect of compact and largely automatic installation make this a practical proposition. However, SBB installations will only become commercially interesting after large-scale solar plants have proved to be feasible. A SBB technology would seem to be rewarding since the combination of modern solar technology with inorganic bacterial oxidation would break down some of the restricting environmental conditions imposed on the solar synthesis of biological products and enlarge the surface area of the world suitable for production of food, energy and materials.

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Amino acid ratios and the correlation of raised beach deposits in south-west England and Wales

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Interest in studies of amino acid racemisation as a framework for Quaternary chronostratigraphy has increased recently¹⁻⁴. These studies have used different materials, but the main two substances have been bone and shell. Different shell genera can have different rates of racemisation⁵, so the use of new genera requires some study of the kinetics of the reactions before the results from one taxa can be compared to another. We report here the successful application of amino acid racemisation studies on the limpet *Patella vulgata* as a basis for correlating the scattered raised marine beaches of interglacial age(s) from south-west England and Wales. We show that the epimerisation of D alloleucine: L isoleucine (D allo: L iso) in *P. vulgata*, provides a useful basis for regional correlation. This mollusc lives attached to rocks above the mean tide level and, hence, spends a good portion of its life living at the ambient air temperature. We suggest that the fossil material may have been primarily exposed to regional atmospheric climate since death, because all available evidence points to sea level being lower than present during much of the past 120,000 yr (ref. 6).

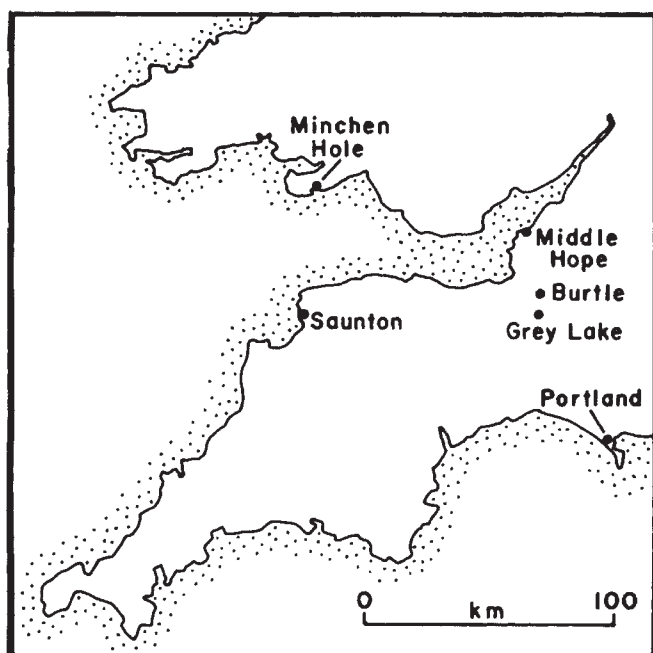
Table 1 Amino acid ratios on *Patella vulgata* (Pv) and *Macoma balthica* (Mb) from sites in south-west UK

Sample no.	Sp.	Location	D allo : L iso (combined)	D allo : L iso (free)
AAL-767	Pv	Modern	$\bar{x} = 0.014 \pm 0.007(3)^* [\text{min. } 0.007]$	—
AAL-786	Pv	Holocene	$\bar{x} = 0.04 \pm 0.006(2) [\text{min. } 0.028]$	$\bar{x} = 0.439 \pm 0.06(3) [\text{min. } 0.379]$
AAL-766	Pv	Holocene	$\bar{x} = 0.04 \pm 0.0015(4) [\text{min. } 0.038]$	$\bar{x} = 0.35 \pm 0.014(2) [\text{min. } 0.34]$
AAL-768	Pv	Skarra Brae	$\bar{x} = 0.119 \pm 0.009 [\text{min. } 0.110]$	$\bar{x} = 0.705 \pm 0.066(5) [\text{min. } 0.608]$
AAL-769	Pv	Burtle Beds	$\bar{x} = 0.099 \pm 0.009(4) [\text{min. } 0.095]$	$\bar{x} = 0.663 \pm 0.068 [\text{min. } 0.59]$
		Minchin Hole		
		Outer Beach		
AAL-771	Pv	Middle Hope	$\bar{x} = 0.101 \pm 0.005(4) [\text{min. } 0.098]$	$\bar{x} = 0.476 \pm 0.088 [\text{min. } 0.38]$
AAL-772	Pv	Portland	$\bar{x} = 0.125 \pm 0.01(4) [\text{min. } 0.123]$	$\bar{x} = 0.461 \pm 0.039 [\text{min. } 0.41]$
AAL-770	Pv	Saunton	$\bar{x} = 0.26 \pm 0.066(4) [\text{min. } 0.2]$	$\bar{x} = 0.629 \pm 0.215(7) [\text{min. } 0.48]$
AAL-839	Pv		(0.081; 0.086; 0.09)†	
AAL-829	Mb	Burtle Beds	$\bar{x} = 0.14 \pm 0.025(4) [\text{min. } 0.12]$	$\bar{x} = 0.499 \pm 0.1(5) [\text{min. } 0.44]$

Sites are shown in Fig. 1 and the data are plotted in Fig. 2.

* No. in sample, when >3 this figure includes reruns.

† Five valves including one radically different ratio, for which three reruns are given in parentheses.

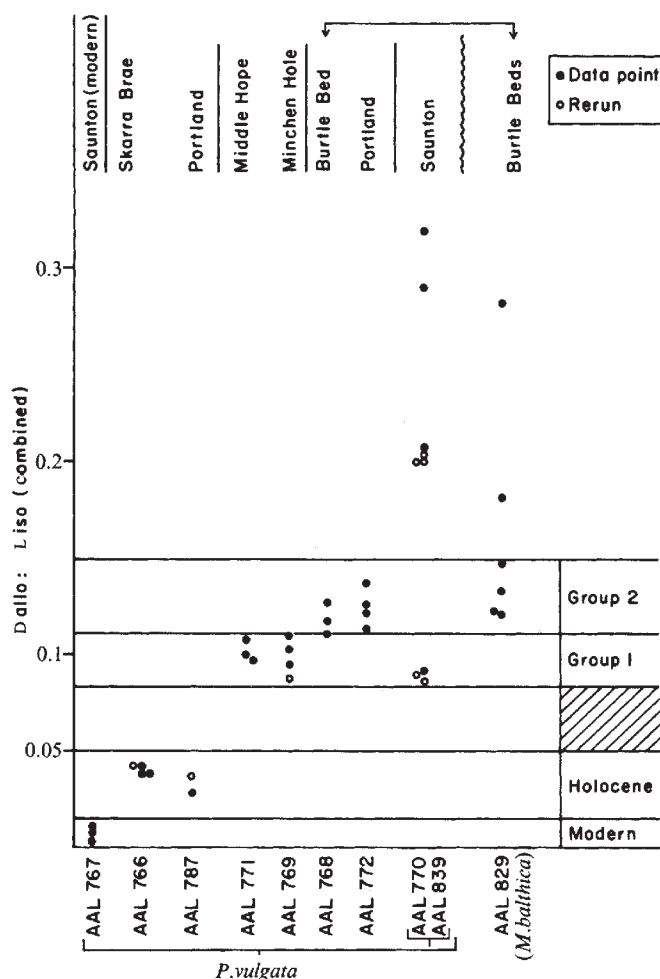
**Fig. 1** Sites of interglacial raised marine beaches.

Three shells were prepared from each site (Fig. 1; Table 1). The shells were first cleaned in 2M HCl, and the cleaned fragments dissolved in 8M HCl. Half the aliquot was then dried under vacuum and rehydrated with a solution of pH 2 containing a 'spike' of the inorganic amino acid, norleucine. This spike provided an internal calibration. The other half was heated in a controlled oven at a temperature of 110 °C for 22 h. It is then dried and rehydrated. These two preparations provided a measure of the free amino acids (those released by a natural diagenesis) and combined amino acids (free plus peptide-bound). The sample was then placed on an ion-exchange amino acid analyser. For most age dating purposes, we relied on the free and combined ratios of D allo : L iso to indicate the relative age of a sample^{1,2}. In addition, we performed pyrolysis experiments on modern shells of *P. vulgata*. Cleaned shell fragments were heated at 144 °C in the presence of 1 ml purified water. At selected times, the samples were removed from the oven, prepared and analysed (Table 2). The results can be compared to similar experiments on valves of *Hiatella arctica*. A feature of the reaction of *P. vulgata* to the heating experiments is the rapid approach of the free fraction to equilibrium (D allo : L iso = 1.35) and the slow epimerisation of the combined D allo : L iso ratio.

A total of eight shell collections have been processed (Table 1; Fig. 2). They consisted of: a modern valve of *P. vulgata* (AAL-767), two samples of Holocene age from Scotland and south-

west England, and five collections from some of the classic raised marine beaches of the region (Fig. 1)^{7,8}. The modern shells show no free amino acids and the D allo : L iso for the combined amino acids varies between 0.007 and 0.02. This ratio is caused by the actual processing of the samples. The modern ratios are significantly different from those obtained from Holocene shells (Table 1) where the D allo : L iso (free) averaged 0.35 and 0.44 and 0.04 and 0.032 (combined) respectively.

Four of the five samples from the raised beaches of south-west England and Wales (Fig. 1; Table 1) fall into two groups with D allo : L iso (combined) ratios of 0.10 and 0.12 (Fig. 2). Repeat analyses of the same preparation (=shell) gave precisions of

**Fig. 2** D allo : L iso ratios for combined amino acids on *P. vulgata* and *M. balthica* from sites in the south-west UK. Data from Table 1.

±5%—smaller than the usual variability between shells from a single stratum (5% to 18%). Analysis of variance (one-way) on the combined D allo:L iso ratios indicates that we can reject the null hypothesis (95% confidence level) that the four samples come from a single population ($F = 8.37$, d.f. 3, 8). The free D allo:L iso ratios (Table 1) do not show an exactly similar pattern and are not considered as reliable an index of relative age as the combined ratio^{10,11}.

The Saunton raised beach site is not so straightforward in the interpretation of the amino acid ratios. Four out of the five shells gave combined ratios averaging 0.26 ± 0.066 which indicates either (1) that higher environmental temperatures affected these shells since death, or (2) the shells are distinctly older than the same species from the other four sites. The fact that one shell gave a typical value for the other raised marine beaches of 0.1 (combined) may indicate that the beach is the product of two distinct high (interglacial) sea levels, a possibility indicated on stratigraphic sources at Minchin Hole Cove, Gower⁷, or that contamination has affected some valves. It is difficult to invoke an explanation for the higher ratios that involves some local temperature perturbation; thus, we favour the second explanation while admitting that more research is required on shell assemblages from the Saunton site. The Portland raised beach and the Burtle Bed samples show somewhat higher combined D allo:L iso ratios than the two other beaches of supposed last interglacial age, taken as equivalent to oxygen isotope stage 5e (ref. 9). It is not yet clear whether the difference reflects a real difference in age or is related to environmental conditions at these sites.

Five valves of *Macoma balthica* were also run from the Burtle Beds (Greylake no. 2). One valve had a cement residue after cleaning and gave a combined D allo:L iso ratio of 0.282. The remaining four valves produced a tight cluster of ratios averaging 0.14 ± 0.025 (Table 1).

Amino acid ratios D allo:L iso for the combined fraction indicate that shells of *P. vulgata* collected from five interglacial sites are either not of the same age, or they have been affected by different temperatures or diagenetic histories since the death of the shells. The Saunton raised beach is clearly the oldest (or most contaminated), although one of the five shells analysed had a ratio similar to those from the Middle Hope and Minchin Hole raised beaches (Fig. 2). These last mentioned sites have ratios somewhat smaller than those determined from the Portland and the Burtle Bed sites. A preliminary conclusion is that the amino acid data suggests the presence in the south-west UK of raised beaches of either two or three different ages. The Portland beach has been regarded as marginally older on altimetric grounds¹². We cannot yet determine whether these represent sea level highs during whole or part of marine isotope stage 5, or part of that 'interglacial' and an earlier interglacial in the case of the Saunton data (Fig. 2). An alternative explanation of our Saunton data is that the majority of the shells are contaminated and that the age of the beach is given by the ratios of 0.09 (Fig. 2).

Correlation between our sites and those reported by colleagues from south and east England¹¹ is made difficult because of the absence of *Patella vulgata* in those sites and because our sites generally lack *Corbicula fluminalis*, the species used most extensively in their study. Our *Macoma balthica*

Table 3 Ratios of *Macoma* spp. from southern and eastern UK* compared to Burtle Beds

Site	Combined D allo:L iso
Burtle Beds	0.14 ± 0.025
Selsey	0.33
Clacton	0.58 ± 0.03
Weybourne Crag } East Anglia	0.8 ± 0.02
Scrobicularia Crag } Sites	0.94 ± 0.005 : 0.998 ± 0.18

* Data from ref. 11.

ratios, however, provide a start to such a correlation programme. Results from assays of *Macoma* spp. from the crag deposits of East Anglia¹¹ (Table 3) show significant differences from our findings. The closest site between our data and that of Miller *et al.*¹¹ is at Selsey, southern England, where a single *M. balthica* gave a combined ratio of 0.33. This ratio is much greater than our results (Table 1), although we note that one sample from our collection which could not be cleaned fully gave a comparable ratio to the *M. balthica* from Selsey. We reject our ratio, however, because we judge it to be contaminated and conclude that the Burtle Beds (Greylake 2) site is characterised by *M. balthica* ratios (combined) of 0.14 ± 0.025 .

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Abrupt end of the last interglacial s.s. in north-east France

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Close study of past interglacials might indicate how and when the present interglacial will end and whether we are heading towards a warming or a cooling^{1,2}. No certain prediction has been possible because of man's interference with the environment. But, we report here that when exploring the records of past temperate intervals, we observed frequent signs of abrupt changes of local environment. In particular, abrupt shifts in forest composition end each Pleistocene interglacial whose record was studied in detail. In Grande Pile (north-east France), the Eemian (s.s.) (oxygen isotope substage 5e) temperate forest was replaced by a pine–spruce–birch taiga within $\sim 150 \pm 75$ yr. These results are based on rich pollen content of continuously deposited laminated gyttja and on the assumption of a constant sedimentation rate during the last 11,000-yr long interglacial. If the area today was affected in a similar way, the relatively fast transition of the environment, and by implication of the climate, would place serious constraints on man.

The continuous pollen-rich deposit from the Grande Pile lake gyttja and peat bog, in southern Vosges (north-east France), is very suitable for this kind of study^{3–6}. It covers the interval from 140,000 yr BP to the present, and includes the last interglacial,

Table 2 Results of heating *Patella vulgata* at 144 °C

Hours heated	Free (D allo:L iso)	Combined (D allo:L iso)
Control (0 h)	—	0.015
20	0.88 (0.66)*	0.194 (0.292)
45	1.25	0.258 (0.66)
80	1.25	0.427
120	1.26	0.44
232	1.23	0.81
286	1.38	0.83

* Values in parentheses refer to *Hiatella arctica*.

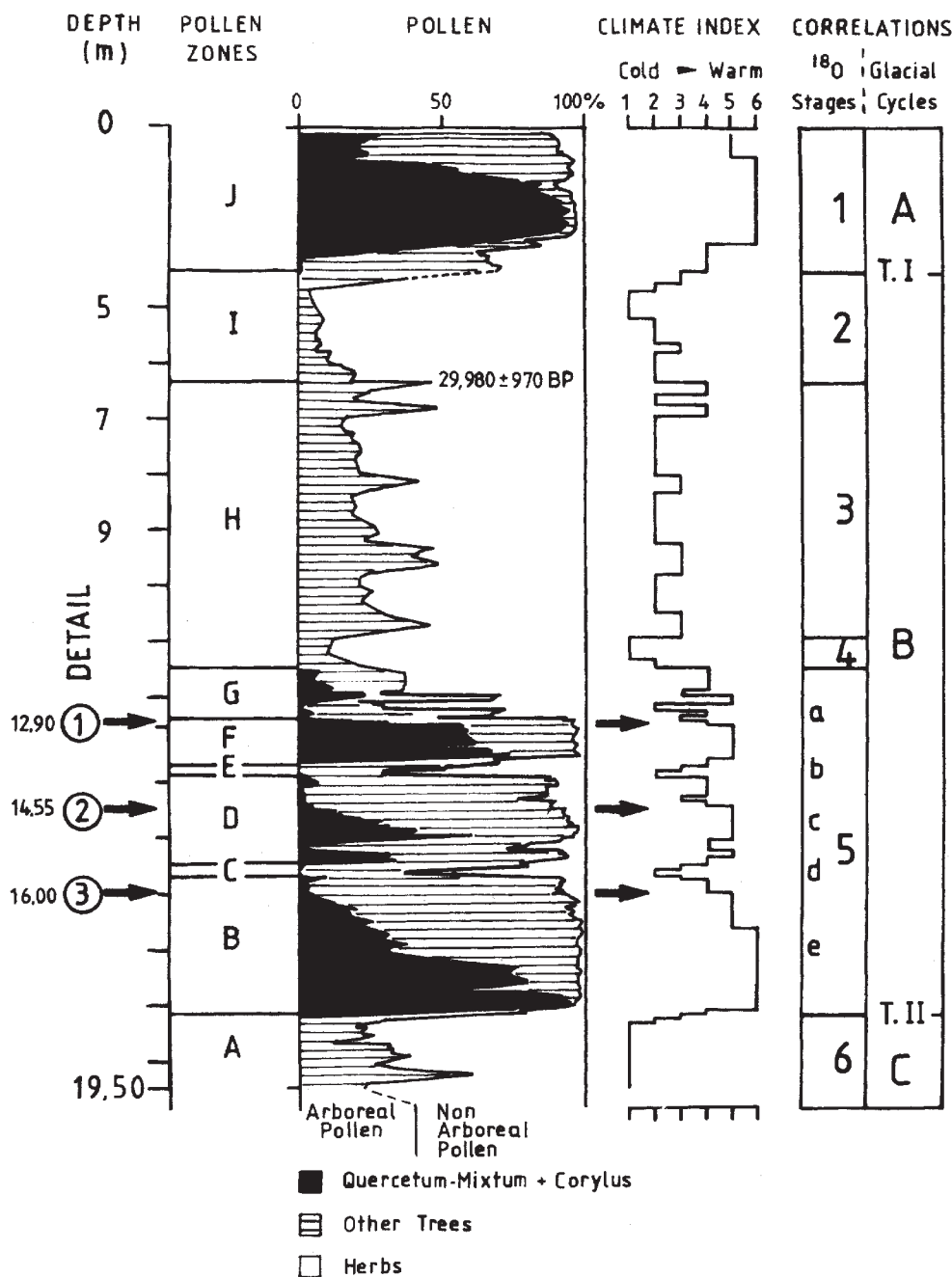


Fig. 1 Grande Pile palynological sequence from the present to 140,000 yr BP. Simplified figure after G.W.^{5,6}; general pollen sequence established with the three cores I, XIV and X; zones A to G from the core X. Between 200 to 500 pollen grains counted at 277 levels; percentages based on total sum. Climate index based on variations in pollen assemblage: 1, tundra; 2, shrub-tundra; 3, open taiga; 4, dense taiga; 5, mixed hardwood-coniferous forest; 6, hardwood forest. ¹⁸O Stages after Emiliani^{27,28} and Shackleton^{29,30}; glacial cycles after Kukla³¹ and Ruddiman and McIntyre³²; T. I. (Termination I) and T. II (Termination II) after Broecker and van Donk³³. 29,980 ± 970 yr BP is the ¹⁴C date Lv. 748. Arrows ①, ②, ③ indicate abrupt climatic changes shown in detail in Figs 2 and 3.

the last glacial and the Holocene (Fig. 1). The climatostratigraphic information is directly comparable to that obtained in northern Greece^{7,8} and in oceanic records^{4,5,9}. Three forest episodes (Fig. 1, zones B, D, F) probably correlate with the oxygen isotope stage 5 (Fig. 1). But, only the lowermost one indicates a climate as warm as the present, and can be correlated with typical pollen profiles of the Eemian, as defined in its type area¹⁰; it is the equivalent of oxygen isotope substage 5e peaking at 125,000 yr BP.

The deposit is finely laminated, so there is no doubt about the continuity of deposition and about the complete lack of post-depositional disturbance.

An abrupt change of vegetational assemblage is observed to end each of the three forest episodes (Fig. 1, arrows 1, 2, 3): all the temperate species retreat, and are even eliminated by a sudden expansion of boreal trees (Fig. 2). A rapid rise of *Pinus*, followed by *Betula* and *Picea*, ends the *Quercus-Carpinus-Corylus* phase of the upper interval (Fig. 2a), the *Quercus-Carpinus* phase of the median one (Fig. 2b), and the *Abies-Picea-Carpinus* phase of the lowermost one (Fig. 2c). A

comparatively abrupt change in vegetation is observed in pollen diagrams from elsewhere, ending past interglacials^{7,10-19}.

As shown at Grande Pile^{5,6}, the Eemian is one of the previous interglacials most resembling the Holocene (climate index in Fig. 1, zones B and J; description in Table 1). The transition from the late-temperate zone to the post-temperate zone²⁰ (Table 1) within the Eemian is rarely demonstrated in terrestrial records because it represents the end of an interglacial. Sediments of this section are often disturbed or removed by glaciation. At Grande Pile, the bog registered the entire Eemian pollen sequence between two glacial episodes (Fig. 1, zones A and C). The abundantly preserved pollen enabled a close study of the critical transition from zone 6a to 6b to be made (Fig. 2c and Fig. 3). A 5 cm-long section of organic sediment was taken from a core of Grande Pile, between two levels previously investigated for pollen, and corresponding to zones Eemian 6a and Eemian 6b respectively, (Fig. 2c). The selected section was prepared for thin sectioning, following the histological technique developed by Johansen²¹. Sections 250 µm thick, taken at 1-mm intervals along the 5-cm long core, were analysed for

Table 1 Vegetational development and zonation of Eemian and Holocene interglacials at Grande Pile (zones B and J in Fig. 1), and comparisons

Vegetational development	Zonation*	Eemian s.s.†	Holocene†
Tundra		Open <i>Gramineae</i> - <i>Artemisia</i> vegetation	
Taiga	IV Post-temperate	7. <i>Pinus</i> , <i>Picea</i> , <i>Betula</i> forest (with development of <i>Ericaceous</i> heaths)	
		6b. <i>Abies</i> - <i>Picea</i> forest (with <i>Pinus</i> and <i>Betula</i>)	
Hardwood-coniferous forest	III Late-temperate	6a. <i>Abies</i> - <i>Picea</i> forest (with <i>Carpinus</i> , <i>Alnus</i> , <i>Quercus</i> , <i>Buxus</i>)	Sub-Atlantic: <i>Fagus</i> forest (with expansion of <i>Abies</i> , <i>Carpinus</i> , <i>Ericaceae</i> , ruderal plants)
		5. <i>Carpinus</i> forest (with expansion of <i>Alnus</i> , <i>Abies</i> , <i>Buxus</i>)	Sub-boreal: <i>Fagus</i> , <i>Corylus</i> , <i>Alnus</i> , <i>Quercetum-mixtum</i> forest (with some <i>Abies</i> and <i>Taxus</i>)
Hardwood forest	II Early-temperate	4. <i>Corylus</i> forest (with <i>Quercus</i> , <i>Taxus</i> , <i>Alnus</i>)	Atlantic: <i>Corylus</i> , <i>Quercetum-mixtum</i> forest
		3. <i>Quercetum-mixtum</i> forest (<i>Quercus</i> , <i>Ulmus</i> , <i>Fraxinus</i> , <i>Corylus</i>)	Boreal: <i>Corylus</i> forest (with <i>Quercus</i> and <i>Ulmus</i>)
Taiga	I Pre-temperate	2. <i>Betula</i> - <i>Pinus</i> forest (with <i>Juniperus</i> and heliophilous herbs)	Pre-boreal: <i>Betula</i> - <i>Pinus</i> forest (with <i>Juniperus</i> and heliophilous herbs)
Shrub-tundra		1. Shrubs (<i>Betula</i> , <i>Juniperus</i> , <i>Salix</i>) and heliophilous herbs	Late glacial

Abies (fir), *Alnus* (alder), *Betula* (birch), *Buxus* (box); *Carpinus* (hornbeam), *Corylus* (hazel), *Fagus* (beech), *Fraxinus* (ash), *Juniperus* (juniper), *Picea* (spruce), *Pinus* (pine), *Quercus* (oak), *Salix* (willow), *Taxus* (yew), *Ulmus* (elm).

* After Turner and West²⁰.

† After G.W.³.

pollen, and the results are presented in Fig. 3. One thousand arboreal pollen grains, at least, were counted at each level in three independent counts; percentages are based on the total sum of pollen grains. The time scale used here is based on comparisons with the work of Müller¹⁴ at Bispingen, in northern Germany. Müller counted the annual varves through part of his record and estimated that the entire Eemian interglacial (equivalent of oxygen isotope substage 5e) lasted <11,000 yr. About 9,000 yr elapsed from the start of the *Betula* zone to the

start of the *Pinus* zone. The same length of time is assumed to separate the beginning of zone 2 from the beginning of zone 6b in Grande Pile, at a constant sedimentation rate (see the Eemian zones in Table 1). A rate of about 6 yr mm⁻¹ thus applies to the sediments of the sampled interval. The maximum error of this estimate may be ±50%.

The pollen data, within the 5-cm section, describe the history of local vegetation as follows (Fig. 3). From bottom to top, four phases can be observed: Zone 6a-top, tree pollen proportion is

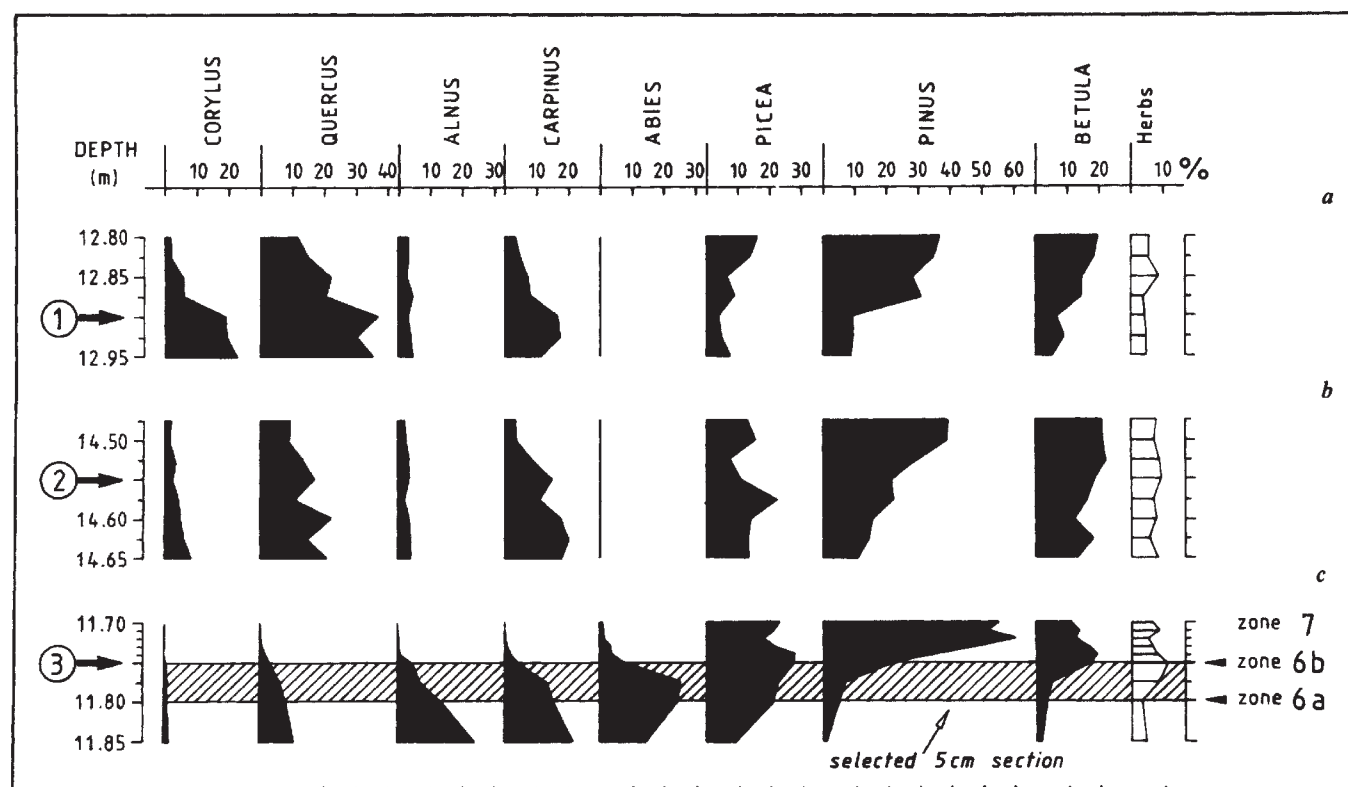


Fig. 2 Pollen counts (% of total pollen sum) across the levels of abrupt environmental change ending the three temperate intervals in Grande Pile shown in Fig. 1. Level 3 was counted in core Grande Pile III instead of Grande Pile X for technical reasons. (Depth level 11.75 in Grande Pile III corresponds to level 16.00 in Grande Pile X, shown in Fig. 1).

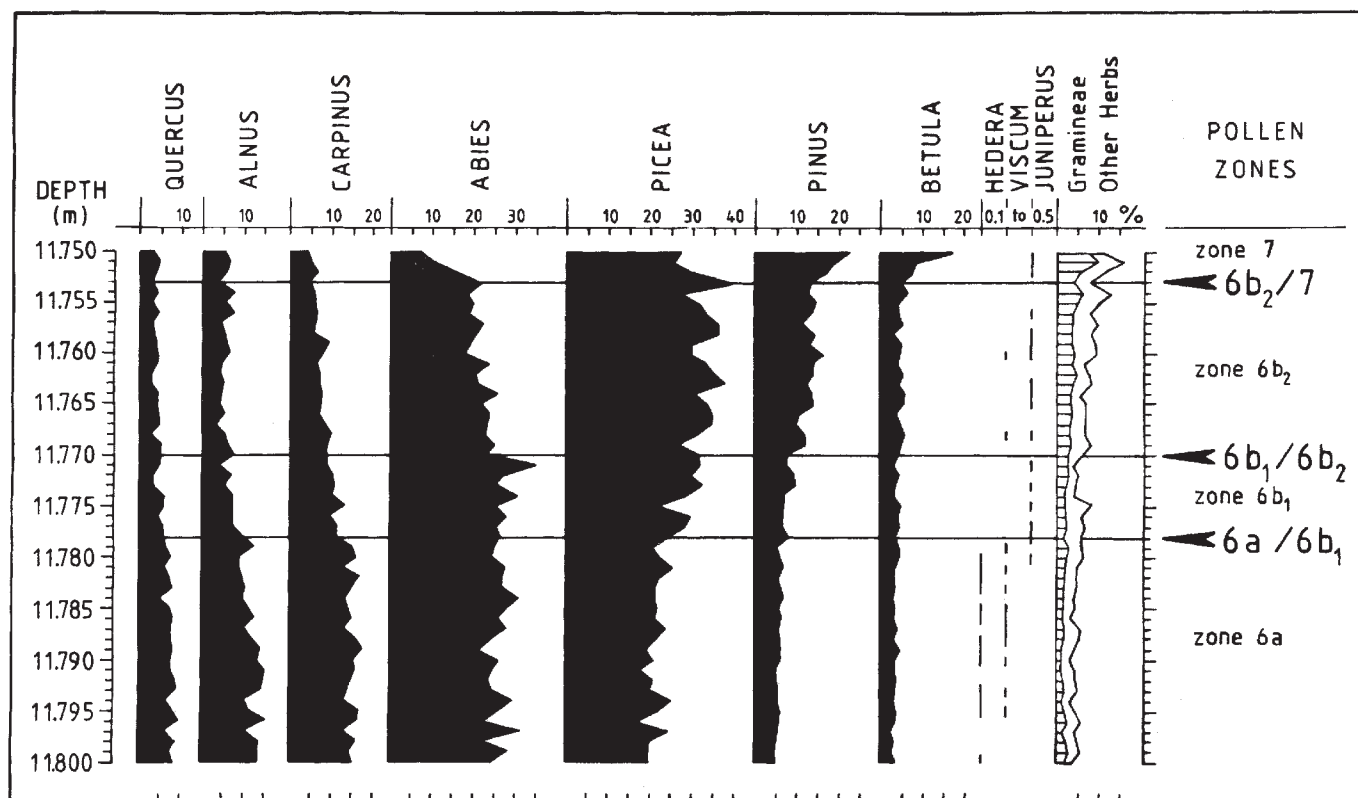


Fig. 3 Selected pollen types in % of total pollen sum. Taken from 250- μ m thin sections at 1-mm intervals within the 5-cm segment shown in Fig. 2c. Estimated deposition rate is 1 mm per 6 ± 3 yr, so that the segment sampled in detail covers 300 ± 150 yr. At each level 1,000 pollen grains were counted.

regular, with little variation, indicating a stable composition of the forest. Zone 6b₁, *Picea* gradually increases, competing with *Abies*: temperate elements decline. Zone 6b₂, *Picea* now clearly dominates over *Abies*: temperate elements are almost missing. Zone 7, Sudden expansion of *Pinus* and *Betula* and abrupt decline of *Abies*. (I assume that all the species involved in the competition existed in the pollination area of Grande Pile, within a radius of about 50 km.) Based on the time scale chosen, the upper part of zone 6a (22 mm thick) had a duration of ~130 yr, zone 6b₁ (8 mm thick) ~50 yr, zone 6b₂ (17 mm thick) ~100 yr, and zone 7 (3 mm thick) about 20 yr. Three relatively rapid changes in vegetation thus appear in the diagram. These are the 6a/6b₁ boundary where *Picea* dominates over *Abies* for the first time. Simultaneously, the temperate deciduous trees (*Quercus* and *Alnus*) decline, even the late-migrating *Carpinus*. Then, for ~50 yr, a close competition between *Abies* and *Picea* takes place, the latter gradually expanding. At the 6b₁/6b₂ boundary, the preceding trends become more pronounced. Now, we can clearly see the dominance of *Picea* and the rise of *Pinus*. The transitional period 6b₂ lasted ~100 yr. The main shift in vegetation apparently took place in only a few years, at the level 6b₂/7. The 6b₂/7 boundary is the best marked in the diagram: in ~20 yr, *Pinus* and *Betula* became with *Picea* the main constituents of the landscape while other trees, even *Abies*, were removed. The landscape is totally modified: the temperate hardwood forest is replaced by a boreal forest, common today in northern Scandinavia between 60 and 65 °N, that is some 15° lat further north than Grande Pile. But, change at the 6a/6b₁ boundary also seems to be important: it marks the irreversible decline of temperate elements (Table 1, zones Eemian 6b and 7). What happened at this level? The *Abies* behaviour on the Alsatian slope of the Vosges, during the very dry summer in 1976, might give one possible answer. *Abies* was seriously damaged by the severe summer drought; it was killed at the low altitudes and only survived in cool high areas. Clearly, repetition of such severe droughts would definitely exclude *Abies* from the Alsatian slope of the Vosges. But *Abies* is not the only species

affected; all deciduous trees show a decline, even species resistant to drier conditions such as oak. The quasi-continuous presence of *Viscum* (mistletoe) which requires relatively high summer temperatures (+15.5 °C, after Iversen²²), and that of *Hedera* (ivy) which requires mild winters (temperatures above -1 °C, after Iversen²²), were ended. At the same time, *Juniperus* became common, a sign of transitions from warm to cold periods. These observations suggest that the change was primarily related to cooling and not to drought.

However, the pollen diagram alone cannot accurately reveal the cause of the change. The pollen curves record local and regional vegetation, and many local or regional factors, unrelated to climate, may modify the landscape^{23,24} (soil structure, soil moisture, local or regional soil variations, competition between species, parasitic fungi and bacteria, destructive insects, diseases). Moreover, change of predominant wind direction may influence the pollen rain. Some pollen may be transported over long distances. For example, in the forest-tundra zone of the Nouveau-Québec, Canada, the pine area has its boundary at 55 °N, but the pollen is carried much further north. Heim²⁵ found 30.8% of *Pinus* in the tundra of Povungnituk, at 60° 34'N. Nevertheless, Heim²⁶ estimated that 60% of the total pollen represents the local vegetation, only 40% resulted from a regional pollen transport.

In Fig. 3, the change during 6a-6b₂ units proceeds gradually, suggesting that the controlling factor was non-episodic. The change at 6b₂/7 boundary is more difficult to explain, because it is so sudden. The continuity of sedimentation excludes any hiatus. The major break in vegetation, marked by the sharp rise of *Pinus* and *Betula*, might be due to sudden death of competitive species with higher climatic requirements. A similar replacement of a temperate forest by a boreal forest is observed at the end of all previous interglacials. It is, therefore, reasonable to assume that the sequence, shown in detail in Fig. 3, is typical, and mainly reveals the impact of global cooling on regional vegetation. This means that regional factors may have had an accelerating or delaying effect locally, and that different

floral assemblages may have responded differently and at different rates to the climate change.

My pollen work shows that, in Grande Pile, the change from a temperate to a boreal vegetation occurred over 150 yr ($\pm 50\%$). The accompanying gradual decline in temperate elements would probably now be hardly perceptible to man, because of the artificial management of many European forests. We thus cannot exclude the possibility that we already live at the beginning of the present equivalent of the terminal interglacial pollen zone, and that we are heading towards a relatively fast, perhaps dramatic, 'borealisation' of West European forests which, some 115,000 yr ago, took < 20 yr.

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Small non-passerine birds of the Lower Tertiary as exploiters of ecological niches now occupied by passerines

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In spite of the rather inadequate sample of past avian populations provided by the chance preservation of fossil bird bones, the increasing accumulation of such material makes it possible to speculate on the composition of early faunas and to consider the apparent relative dominance of major groups, such as passerines and non-passerines, in earlier geological periods. An examination of the range of species that we now know occurred in the Lower Tertiary of Britain appears to confirm that passerine birds were very scarce and that their great adaptive radiation had not taken place. Their ecological niches were seemingly occupied by small non-passerine land birds, species of this size appearing proportionally more numerous than at the present day.

Recent studies of British Lower Tertiary birds, involving both new and previously-described (but not always correctly identified) material, have greatly increased the number of species known. The total of British Lower Eocene species from the Woolwich and Blackheath Beds and London Clay of the London Basin, and the London Clay of Bognor Regis, Sussex, has risen from 8 to 31 (ref. 1); six species have been described from the Middle Eocene Bracklesham Beds of the eastern Hampshire Basin²; the Upper Eocene avifauna from the Barton Beds of the Hordle area of the Hampshire Basin has increased from 9 to 13 (ref. 3), and five species have been described from the Lower Oligocene Hamstead Beds and Bembridge Marls of the Isle of Wight⁴. Further undescribed species are known from the Lower Eocene London Clay of Essex and the Middle Eocene Barton Beds of the Hampshire Basin. Sixteen orders and twenty-three families are now known to be represented in the Eocene and Lower Oligocene of Britain, and by the standards of modern temperate regions this constitutes a reasonable sample of a local avifauna at this taxonomic level, but lacks the complex adaptive radiation of passerines.

As might be expected from these water-borne deposits, most of the species are waterbirds, and include seabirds. The bone fragments of these species are mostly of moderate to large size and are referable to forms which, for the most part, are within the size range of Recent species in the taxa involved, but tend to be towards the upper end of these ranges. This is not surprising because large bones are more likely to be preserved and be noticed.

Collectors, when working in the field, tend to look for material within a particular size range, and there is apparent evidence of this type of 'search image' bias in the British Upper Eocene material. The earlier collections of Upper Eocene avian specimens³ consist almost entirely of bones or bone fragments more than 23 mm long and fairly thick; but R. Gardner, while searching for the remains of small mammals in the same Barton Bed deposits that yielded the earlier material, recently collected several fragments mostly less than 10 mm long and 2–3 mm thick. Because these included bones of a new small wader and small passerine-type bird, the remains of small species have clearly been overlooked, and a higher proportion of such species is to be expected from future collecting. In spite of the mainly marine and estuarine origins of these Early Tertiary deposits in Britain, the terrestrial avifauna which has been preserved in them is surprisingly large. The term terrestrial is used here to indicate both cursorial and arboreal land birds, as opposed to species from marine, littoral or lacustrine habitats. The size distribution of these terrestrial non-passerines differs from that of the birds as a whole, as the following list of British forms indicates. In it, except where noted, the birds are from the Lower Eocene. The names of some Recent genera and species are given in parentheses to indicate relative sizes of the fossils concerned.

Falconiformes: one large (buzzard, *Buteo*), one medium-sized from the Middle Eocene (kite, *Milvus*), one very small (pygmy falcon, *Microheirax*); Galliformes: two large from Middle Eocene and Lower Oligocene (junglefowl, *Gallus*), one medium-sized (common partridge, *Perdix perdix*), four small, with one from the Middle Eocene, (from two-thirds partridge size to size of quail, *Coturnix*); Columbiformes: one very small (diamond dove, *Geopelia cuneata*, smallest Recent pigeon); Psittaciformes: one medium-sized (Senegal parrot, *Poicephalus senegalus*); Strigiformes: one a little smaller than the little owl, *Athene noctua*; Cuculiformes: one large musophagid, three tiny cuckoos (bronze cuckoos, *Chrysococcyx*); Coraciiformes: one medium-sized (song thrush, *Turdus philomelos*).

Thus, by comparison with the size ranges of the Recent species in the taxa concerned, and in circumstances where there is a tendency for large rather than small species to be collected, of the eighteen species from these seven orders four were large, four medium-sized, and ten small to very small. In this preponderance of small forms the early avifauna differs markedly from Recent taxa. Although about a third of Recent owl species are small, and about one-sixth of phasianid gamebirds; only about

14 of 131 cuckoos, 11 of 286 pigeons and nine of 263 diurnal birds of prey come into the latter category. Because most of the small fossils were found at the same sites as medium and large ones there is no suggestion that selective transport of material in a marine environment before final deposition has influenced the size range of specimens; and on present evidence these early terrestrial birds show an exceptionally high proportion of small species.

In Recent terrestrial avifaunas most small birds are passerines, Passeriformes. They form 65% of the terrestrial avifauna of Europe; and in a world study of Recent avifaunas, Slud⁵ found an overall ratio of 2:1 between passerines and non-passerines in terrestrial faunas, with a slightly higher ratio in forested than in non-forested regions.

The Passeriformes are very poorly represented in the Lower Tertiary. One species has been described from the Lower Eocene of Britain¹ and another has been identified provisionally from the Upper Eocene. Fisher⁶ lists one Lower-to-Middle Eocene species from North America which Feduccia⁷ has re-identified as piciform, and four Upper Eocene passerines from France which Brodkorb⁸ regards as *incertae sedis*. Brodkorb has also questioned the existence of any passerine birds before the Miocene. At best passerines would form only 10% of the known terrestrial avifauna of the British Lower Tertiary. Because the range of forms includes very large and very small birds, representatives of families in which the present species are almost entirely arboreal, and a number of taxa in which the Recent birds have poor powers of flight, there is no reason to suppose that passerine species, if present at that time, would be any less likely to occur as fossils than these other small species.

Assuming that the known fossils are a reasonable sample of the British Lower Tertiary avifauna, then that avifauna differed from all present ones in having a severe deficiency of passerine species, and in a preponderance of small species among the other orders. Most of these small non-passerines probably fed on seeds or insects and other small organisms, thus occupying the main types of feeding niches of Recent passerines. I suspect that the two differences are linked, and that non-passerines originally occupied feeding niches available for small birds, and were later excluded from these by the adaptive radiation of the passerines. This would in turn have affected the opportunity for survival and adaptive radiation of small non-passerine species.

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Complex systems which evolve towards homeostasis

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It is still not well understood how large complex systems (such as ecosystems) come into being or how they persist over long periods of time, although recent studies^{1–5} have clarified certain aspects of the associated stability problems. The occurrence of such systems presents no problem if a teleological assumption is valid—that is, if the variables may be presumed (or compelled) to adjust their mutual interactions in advance, to achieve the desired persistence. But when no such ‘foresight’ is credible, a

real problem emerges; how a given variable can interact will generally be constrained by factors internal to it, independent of the ‘needs’ of other variables or of system stability. This consideration has prompted studies of ‘randomly-interacting’ system models, and interesting results have emerged^{1–4} concerning the stability of such models—that is, their capacity to return to equilibrium after a fluctuation. However, the question remains: how did such a collection of randomly-interacting entities arrive at an equilibrium in the first place? We present here results from a simple model using mutually interacting variables. We show how, even on assumptions which make large complex and persistent systems highly improbable initially, they can nevertheless be expected to arise in profusion in the normal course of time development.

To fix ideas, our model is presented as an ecosystem but the field of application is much wider. Such an extension is possible because we have not included specifically biological mechanisms; thus, from an ecological as from any other particular viewpoint, the model is bare and lacking in realistic detail. This is deliberate; we seek the consequences of this very general model itself—the ‘mathematical’ consequences—rather than those peculiar to the one field of application.

We consider, then, a collection of M species in which the (i)th has an intrinsic increase rate r_i (positive for a producer, negative for a consumer). Its interactions with the other species are fully described by $(M-1)$ ‘interaction constants’ A_{ij} , and A_{ii} describes its self-interaction. The time-dependent variable $N_i(t)$ is taken to measure its population, population density or biomass.

A simple form for the system’s equations of motion was chosen, sometimes called the generalised Lotka–Volterra (GLV) equations, but here referred to as the multispecies quadratic (MSQ) model. (This avoids confusion with other definitions of ‘GLV model’.) These equations are;

$$dN_i/dt = N_i \left(r_i + \sum_j A_{ij} N_j \right), \quad i = 1 \text{ to } M \quad (1)$$

To investigate the time behaviour, we proceed as usual (see for example ref. 5). The equilibrium values N_i^* are found as the solution of the matrix equation

$$AN^* = -r \quad (2)$$

The local stability/instability of the system about these equilibrium values is then determined by the absence/presence of eigenvalues of S with a positive real part, where $(S)_{ij} = N_i^* A_{ij}$. However, this stability character has no meaning unless the equilibrium point is ‘feasible’, that is, unless all the N_i^* are positive³. A system which is stable about a feasible equilibrium will be called ‘homeostatic’.

In computer studies on this model, we have taken the A_{ij} ($i \neq j$) as non-zero with probability C (the connectance), and then of constant magnitude g . The self-interaction coefficients A_{ii} and the increase rates r_i were chosen as having magnitude 1. By allowing the non-zero A_{ij} and the r_i to be positive or negative with equal probability, a ‘random ensemble’ was generated for study.

Initial results for M ranging from 2 to 10 have already been reported⁵, and are briefly summarised here. In this model, despite its ecological poverty, the subset of homeostatic systems is reminiscent of real ecosystem behaviour—in particular, in the popularity of predator–prey interactions ($A_{ij} < 0$, $A_{ji} > 0$, $r_i > 0$, $r_j < 0$), the fraction of consumers tolerated ($r_i < 0$) and the necessity for nearly all species to be self-regulated ($A_{ii} < 0$). In all these ecosystem-like properties, the simple requirement of homeostasis picks out a subset very different from the rest of the random ensemble.

But in one important respect, the model fails badly: it gives far too low an estimate of the chance that such a random collection of species will be homeostatic, that is, persistent. This chance lies below 2^{-M} , or, for as few as 20 species, less than one in a million. Thus other mechanisms must be considered, if the model is to yield a plausible likelihood for the occurrence of large systems.

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Table 1 Results of species elimination

	Elimination method	
	Random	Natural selective
Maximum no. of eliminations required for homeostasis	50	29
Mean no. of eliminations required for homeostasis	46.7	25
Fraction of sample yielding a non-trivial homeostatic system	24%	100%
Fraction of feasible systems stable	100%	100%
Largest homeostatic system resulting	4	29
Smallest (non-trivial) homeostatic system resulting	2	21
Mean ratio of connectance in homeostatic set to that in random ensemble	0.80	0.99
Fraction of eliminated species which were consumers	0.50	0.826
Maximum fraction of consumers in a homeostatic system	0.33	0.25
Minimum fraction of consumers in a homeostatic system	0.00	0.11
Mean fraction of consumers in a homeostatic system	0.20	0.165
Ratio of mean no. of eliminations to initial no. of unfeasible species	1.82	0.99
Final range of values of g	0.88–1.25	0.33–0.39

Sample of 25 from a random ensemble of self-regulated species collections ($A_{ij} < 0$ for all i), with $M = 50$ (25 consumers, 25 producers), $C = 0.2$, $g = 0.25$ initially. Starting with inhomeostatic systems of 50 species, species are successively eliminated and the interaction strength 'scaled' (see text), until a homeostatic system is achieved. In the random elimination method all species have equal chance of removal. A 'non-trivial' system is one containing 2 or more species.

One might think that specifically biological mechanisms now need to be invoked—for example, the evolutionary processes which lead to what Wilson has called 'adaptative equilibrium'⁶. However, we report here a study which invokes no such 'external' consideration, but derives the higher likelihood desired from the bare model itself. This is achieved as follows: Either of the defects which imply inhomeostasis—unfeasibility, or instability—can be expected to result in the eventual disappearance of one or more species, so that the original collection does not persist in time. Furthermore the elimination of species might proceed until only a handful of species remain, perhaps none at all; but alternatively, the residual system might be comparable in size to the original collection. We have tested for this latter possibility, generating random assemblies as described above, but imposing $A_{ii} = -1$ always to ensure the possibility of homeostasis⁵.

Starting with a random (almost always inhomeostatic) collection of 50 species, if it is unfeasible we eliminate the species with the most negative equilibrium value N^* . A second criterion was adopted to choose the species most likely to disappear from a feasible but unstable collection; we omit this criterion, for reasons evident below. Both criteria are intuitively appealing, and can be supported. The resulting collection of 49 species is then examined. If it is still inhomeostatic, another species is eliminated using the same criteria; this procedure is repeated until a homeostatic system is obtained, or all species disappear. (On reducing the number of species, say to M' , we increased the interaction parameter g to g' so that $(M' - 1)^{1/2}g'$ kept its initial value $(M - 1)^{1/2}g$. For the rationale of this 'scaling' (note that it makes homeostasis harder to achieve in the smaller system) see refs 2 and 5.)

Table 1 gives detailed results for a sample of 25 assemblies treated as described. It will be seen that inhomeostasis, whether initial or final, always stemmed from unfeasibility; feasible systems were always stable. (Other evidence for this finding in

the kind of parameter range studied already existed, though for a less general model consisting of producers only³.)

The major result is that no assembly 'collapsed' to fewer than 21 species, and one retained 29. The mean number of species remaining in the final homeostatic system was 25, or half the original number.

The large size and complexity of the final system depended on the elimination being 'natural-selective', that is, designed to mirror the actual result of development in time. This is readily seen from Table 1, by comparing the results with those of 'random' elimination, where such a rationale was lacking; the largest homeostatic system then achieved held only four species, and most of the species had no interspecific interactions.

In the homeostatic systems arrived at by selective elimination, the distribution of interactions was examined; it was not detectably different from that of homeostatic systems obtained in random choice from the ensemble. Moreover, the connectance C remained essentially unaltered in the passage to homeostasis. Thus, if we want to construct a typical large homeostatic system, of size $\sim M$ and connectance C , it is quicker to generate an assembly (almost certainly inhomeostatic) of $2M$ species with connectance C , and let it achieve homeostasis by natural-selective elimination. The alternative is to continue generating assemblies of M species until a homeostatic one appears; we estimate that, for M as small as 25, the elimination method is quicker by a factor exceeding 500,000.

It is evident that, in the natural evolution of such assemblies if randomly generated in time, almost all large, multispecies systems will be those arrived at through elimination. Moreover, they will appear with far greater speed, and thus with far greater likelihood, than would be suggested by the (tiny) proportion of the random ensemble that they constitute.

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Heart attacks and geomagnetic activity

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Malin and Srivastava¹ reported a remarkable correlation between daily variations in the geomagnetic field strength and daily admissions to the cardio-thoracic wards of hospitals in Hyderabad and Secunderabad, for cardiac emergencies, during 1967–72. We have now carried out a similar enquiry in the West Midlands region of the UK for the years 1969–70, but were unable to confirm the Indian results.

The medical data consisted of daily admissions for acute myocardial infarction to all hospitals in Warwickshire, including Coventry, Birmingham, Solihull and Sutton Coldfield, during a period of 720 days beginning 1 January 1969. The data were extracted from the Hospital Activity Analysis records of the West Midlands region. The geomagnetic data consisted of the daily sums of the 3-hourly 'Kp-values' obtained from catalogues of recordings taken in the UK². Fluctuations at three geographical sites were reported and agreed closely; the data used in the analysis were those recorded at the station at Hartland,

UK. There were 6,298 hospital admissions over a 720-day period, a mean of 8.75 per day. The 'Kp-sums', also consisting of integer values, averaged 15.73 per day. Analyses were carried out with both the raw data (n) and logarithmic transformations ($\log_{10}(n+1)$).

Same-day correlation coefficients were calculated between the two values within each of 24 successive 30-day periods. Serial correlations were calculated, separately for geomagnetic activity and for heart attack admissions, between pairs of readings separated by 1, 2, ... 31 days. Finally, over the full 720-day period, correlations were calculated between geomagnetic readings on day 0, and hospital admissions on days -15, -14, ... +14, +15 days.

The results were qualitatively similar for the raw and the log-transformed data. Results reported below refer to the log transforms. The serial correlations of the admission data gave significant values (>3 s.e. from 0) at 7 days ($r = +0.15$), 14 days ($r = 0.16$) and 21 days ($r = 0.12$). A complex day-of-week variation over the full period ($\chi^2_6 = 67.8$), mainly characterised by a deficiency of admissions on Sundays, confirms the validity and demonstrates the origins of this finding. The serial correlations of the geomagnetic data gave significant positive results (>3 s.e.) at 1/2 days ($r = 0.50$ at 1 day), 16/17/18/19 days (0.17 at 17 days) and 25/26/27/28 days (0.21 at 27 days). The 27-day cycle represents the period of rotation of the Sun. None of the 720-day-period correlations with intervals of -15, ... 0, ... +15 days showed significant positive or negative correlations; the greatest absolute value was $r = +0.06$ (1.46 s.e.). None of the intra-30-day-period same-day correlations between medical and magnetic data gave a significant value, either positive or negative.

Despite the fact that our methods and materials were sensitive enough to detect serial correlations within each of the individual data sets, our examination failed to confirm the Indian observations. Although there is no clear explanation of the difference between the two investigations, three hypotheses should be considered: (1) heart attacks may have different aetiologies in the two countries, (2) the two sets of electrocardiographic equipment may differ, the Indian equipment being susceptible to external magnetic fields, thus affecting the relative likelihoods of different diagnoses, (3) temporal fluctuations in the Indian medical data, with a period differing from our own 7-day cycle, and due to different social customs, may have interacted coincidentally with one or other of the periodic variations observed in the geomagnetic field.

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Perception of illusory movement

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Intensive studies of visual illusion have rarely shown examples of polymorphic responses¹⁻³. We show here that, using figures consisting of stripes shaded from dark to light, arranged in repeating sectors, an illusion of movement can be induced in about 75% of observers when viewed peripherally. The responses of the viewers fall into four categories. This polymorphic response suggests a genetic origin.

Figure 1 shows the figures used during the experiments. The four categories of response into which viewers could be divided were: (1) N, seeing no movement, or a slight, inconsistent, jerky motion; (2) DL, seeing smooth continuous movement in the

direction from dark to light shading; (3) LD, seeing smooth, continuous movement in the light to dark direction; and (4) V, sometimes seeing smooth, continuous movement in the DL direction, sometimes in the LD direction. The individuals of the N class who see a slight, jerky movement consistently see this in the DL direction. A sample of 678 subjects showed 24.9% N, 59.0% DL, 6.5% LD and 9.6% V. The subjects were tested with two figures in which the shaded sectors were arranged in a spiral and the polarity of the shading was opposite. Sensitive subjects see the illusory movement in opposite directions in the two figures. Care was taken not to bias the responses of the subjects who were simply asked to give their responses to the figures, no mention being made of motion, rotation, and so on. Reaction to the figures in repeated tests separated by intervals ranging up to 2-3 years is remarkably constant, that is, the classification has a high repeatability.

The polymorphism of response suggested a genetic explanation and, therefore, a parent-offspring survey was made of individuals in 83 families. The LD and V classes were grouped to give the data shown in Table 1. There are no differences between sexes nor between reciprocal matings, and the data have been grouped over sexes and reciprocal matings. If we examine the types of matings from $N \times N$ to $N \times DL$, to $DL \times DL$, to $DL \times LD-V$, there is a progressive decrease of the frequency of N progeny. There is a clear parent-offspring correlation, indicating that there is either a strong genetic causation or an equally strong familial effect of common environmental factors.

A number of twins were tested to allow separation of genetic and environmental factors. Concordance was computed as the number of twin pairs having the same response divided by the total number of twin pairs. The concordance for MZ twins (29 pairs) was 0.90, for DZ twins (41 pairs) was 0.56, and for non-twin sibling pairs was 0.53 (213 pairs). The concordance for unrelated pairs was 0.40. The differences between MZ and DZ twins are significant, indicating that there is a fairly strong genetic component. Clearly, more data are needed, but the agreement between the parent-offspring and the twin comparisons indicate that the illusory-movement perception (which we are terming the 'escalator' illusion) offers considerable promise for further detailed studies.

If our data are sub-divided by academic disciplines it is apparent that the incidence of the escalator effect differs between academic disciplines. Faculty and graduate students of three departments were tested: Fine Arts, 4.7% N (42 tested), Biological Sciences, 22.5% N (40 tested), Psychology, 56.2% N (42 tested). The Biological Sciences department does not differ from the general expectation of 24.9% N. The other two departments differ significantly from each other, and from the general expectation. Tests of progeny of Fine Arts and Psychology faculty gave the following results: Fine Arts, 5.9% N (17 tested), Psychology, 35.0% N (20 tested). The regression of the progeny towards the population mean of 25% agrees with the genetic determination of the character. These results support the surprising conclusion that genetic differences in

Table 1 Parent-offspring survey of individuals of 83 families

Parental mating type (and no. of matings)	Response type of offspring (%)		
	N	DL	LD
$N \times N$ (10)	18 (52.9)	12 (35.3)	4 (11.8)
$N \times DL$ (28)	22 (28.6)	43 (55.8)	12 (15.6)
$DL \times DL$ (33)	5 (5.1)	77 (77.8)	17 (17.2)
$DL \times LD-V$ (9)	1 (3.8)	15 (57.7)	10 (38.5)
$N \times LD-V$ (2)	1 (25.0)	0	3 (75.0)
$LD-V \times LD-V$ (1)	0	3 (75)	1 (25.0)

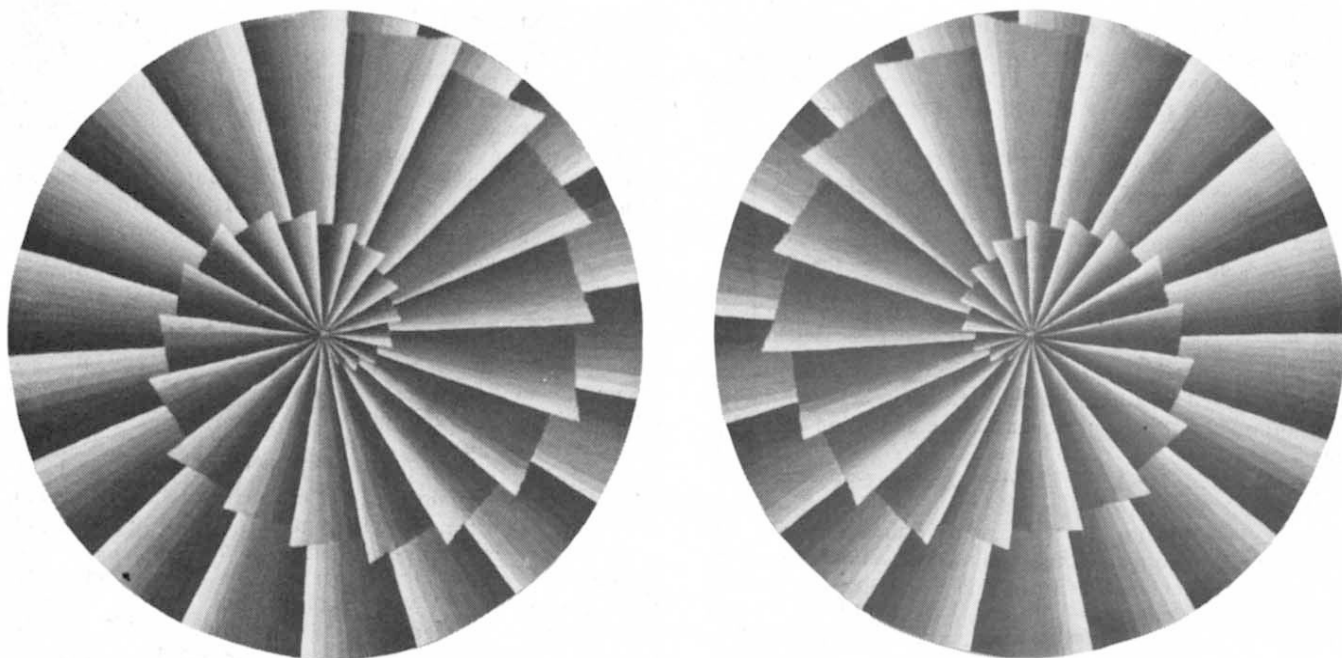


Fig. 1 The two illustrations presented to observers.

sensitivity to the 'escalator' figures are involved in career selection. We have identified in our sample of Fine Arts undergraduate students, five who transferred out of the programme. All were N ($P = 0.001$). Presumably the complementary process occurs in the Psychology programme. Genetic determinism of a sociological polymorphism is known—similar differences between career groups can be found for colour blindness.

'Escalator' illusion is a feature of peripheral vision not occurring within 5° of the point of fixation when seen at distances of several feet. The possibility that this phenomenon is due to small polarised eye movements is discounted, (1) by seeing simultaneous rotation in opposite directions in spiral figures, (2) by seeing simultaneous movement in opposite directions in parallel and rectilinear figures, and (3) by seeing the simultaneous different movements continuously whilst making fairly large eye movements.

Tests were made to determine whether real rotation would suppress or enhance the rotation of the figures induced by the 'escalator' effect. The two figures were mounted side by side and rotated at the same rate. Subjects viewed these figures peripherally centering their vision mid-way between the figures and below them. The two disks were concordant, having their DL shading in the same direction, or discordant, one disk having the DL shading in the clockwise direction whereas its neighbouring disk was shaded DL in the anticlockwise direction. It was found that if the rate of rotation is slow (0.68 r.p.m.) then DL subjects differ in their perception of the rates of rotation of the disks, depending on whether they are concordant or discordant. They see the concordant disks as rotating at the same speed in 91% of trials. Conversely, they see in the discordant disks, one disk as rotating faster than the other. The disk seen as rotating at a faster rate is the one in which the DL shading is in the same direction as the real rotation. This was reported in 68.0% of trials. If the real rotation is reversed, then the identification of the faster disk is also reversed. N subjects gave quite different results in their perception of the discordant pair of disks, reporting the expected difference in apparent rate of rotation in only 18.0% of trials. At slower rates of rotation the agreement of results with expectation increased to 90.0% in DL subjects and to 59.7% in N subjects. At very slow rates of rotation in the range of 0.1–0.4 r.p.m., DL subjects note for the discordant pair of disks that the disk in which the DL shading is in the opposite direction to the real rotation, appeared to be

stopped whereas the adjacent disk was still perceived as rotating. Reversing the direction of the real rotation reversed the identification of the disk that was not rotating. N subjects noted the same apparent cessation of rotation only at lower rates of rotation 0.12 r.p.m. and with less consistency. It is apparent that our original test procedure does identify a real difference of visual perception, with the difference being of degree: N subjects are not 'blind' to the 'escalator' effect, but have a much lower sensitivity than DL subjects.

It is rather surprising that the intensive studies of visual illusions^{1–3} have rarely identified examples of polymorphisms but a possible explanation of this is that such studies have involved refining figures until they are 'strong'; that is perceived by all subjects in all conditions. If figures are, instead, refined to maximise discrimination, then it is possible that polymorphisms of visual perception will be found to be as general as polymorphisms of other characters.

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Underwater behavioural thermoregulation in the adult stonefly, *Zapada cinctipes*

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Some adult insects survive extremely low temperatures using various physiological mechanisms^{1,2}. Here I report that the adult stonefly, *Zapada cinctipes* (Banks) (Plecoptera: Nemouridae), behaviourally thermoregulates at subzero night temperatures by entering the water.

The study site, a small pond formed by an artificial diversion of Sagehen Creek, was located in the Sierra Nevada Mountains 9 km north of Truckee, California ($39^\circ 25' N$, $120^\circ 12' W$, elevation ~ 2.0 km).

After typical³ midday (11.30 a.m.–2.30 p.m.; 13.0–25.0 °C) aerial or surface oviposition (no species of stoneflies in North America have ever been observed to oviposit underwater⁴), a female would generally fly to the centre of the pond and land on the ice. She would then walk to the south-west edge of the ice, crawl into the water and awkwardly swim ~0.5 m to within 0.25–0.50 m of the shaded shoreline. Next, she moved beneath the water surface by grasping pine stems and branches protruding above the water. (An adult possesses two pairs of cervical gills and these, in addition to being partially enveloped in a film of air, may enhance underwater oxygen diffusion.) A descending female would wave each antenna in front of her in an apparent searching pattern, although contact was rarely made with the object used for descent. This descent continued to a depth of 15–20 cm, where she remained relatively motionless in the clear water for periods of 20–60 min. (Females placed underwater in wire cages in the same location and raised to a point just below the water surface for inspection at 5-min intervals lived as long

adults were more commonly observed to land in open areas along the shoreline and seek refuge in rock crevices and beneath leaf debris (previously inaccessible because of snow cover), where they remained until morning. For the period 4–24 April, mean minimum night air temperature was ($\bar{x} \pm \text{s.e.}$) -6.2 ± 0.7 °C, and the higher mean minimum water temperature 8.6 ± 1.0 °C.

To test the effect of temperature on survival, 12 individuals collected on 8 April were randomly divided into two groups. The first group was placed in a $0.5 \times 0.2 \times 0.1$ -m wire cage and half submerged in the pond. The other six were placed in a similar cage, but kept along the shoreline and partly covered against solar overheating. Pond drinking water was renewed daily for the latter group to control for differential mortality from desiccation; gut analyses of both groups (all guts were empty) confirmed an initial assumption that food was made unavailable by caging. Consequently, mean longevity of the first group ($\bar{x} = 8.5$ d), with access to the pond, was significantly greater than the second group ($\bar{x} = 1.7$ d), which was exposed to subzero night air temperatures ($P < 0.005$; Mann–Whitney test).

An additional field experiment was carried out on 21 April 1979 to measure thermal avoidance more directly. To preclude possible light entrainment, this experiment was carried out at times when underwater behaviour has never been observed. (Testing times, air temperatures and water temperatures for both trials were: 10.20–11.05 a.m., 15.0–16.1 °C and 10.0 °C; 1.05–1.52 p.m., 18.0–19.5 °C and 10.4 °C.) The apparatus consisted of a $14.0 \times 5.4 \times 14.5$ -cm Plexiglass chamber half filled with pond water and submerged in the pond to this same level. Two newly collected females per trial were initially positioned 3.5 cm above the water on pine stems extending the height of the chamber. The top, made of paired sheet aluminium baskets filled with dry ice and separated by an insulated sliding thermometer, was then sealed against CO₂ leakage. At ~6–12 °C, responses consisted of localised repositioning and antennal tip bending and shaking alternately towards the top of the chamber and the water surface. At 4.8–6.1 °C, all stonefly adults moved completely underwater (Fig. 1) for 15–42-min intervals. Adults left the water when air chamber temperatures approached ambient.

A single female submitted to the same experiment at 3.00 p.m., but without access to water, went into a torpor at 3.5 °C. If immediately removed from the chamber and warmed, she recovered and seemed to exhibit normal locomotory behaviour. But, when left at the same temperature for 2–3 min she did not recover.

It seems important that at high altitudes where daily air temperatures fluctuate greatly⁵ and winter conditions are severe, endothermic or ectothermic regulation is used by adults as an adaptive survival strategy. Because endothermy for small insects is too energetically costly⁶, regulation of body temperature by stonefly adults moving to a relatively constant thermal environment (a pond or lake) may be of considerable evolutionary significance. Such a hypothesis is not without support, for in nearby Lake Tahoe both nymphs and adults of the stonefly, *Capnia lacustra*, have been found to live at depths of 60–80 m (refs 3, 7). Although the behavioural flexibility exhibited by such species seems rather remarkable, limits imposed by poor dispersal abilities^{3,8} must be seriously considered in the light of man's increased impact on these faunal refuges.

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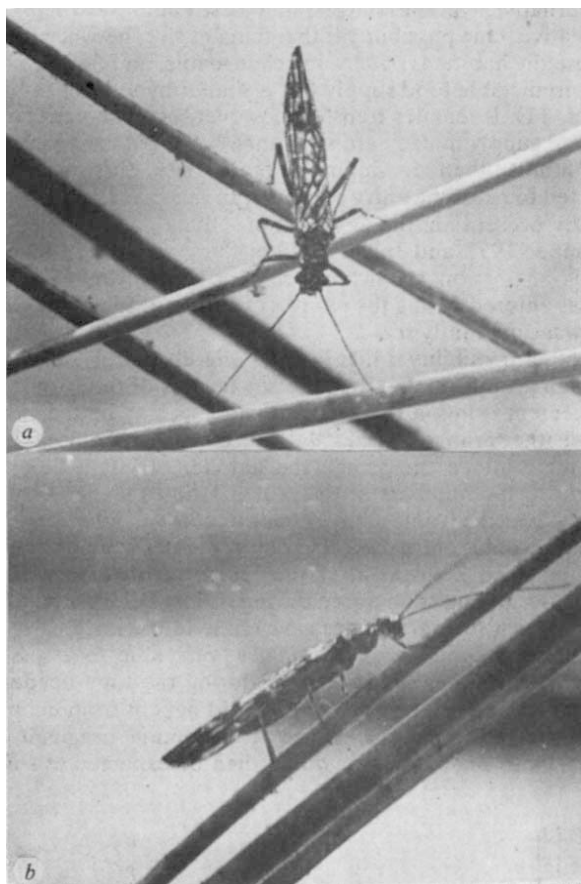


Fig. 1 a, b, Underwater views of an adult female *Zapada cinctipes* (Banks) after submerging at 5.0 °C air temperature ($\times 2.5$).

as 80 min.) A female would then climb to the surface and float for 1–5 min before submerging again. No females collected before submerging were ever observed to be extruding egg masses. This procedure was repeated continuously up to 5 h after sunset, even after a thin layer of ice had sometimes re-formed on the surface. The outlines of air spaces could be seen immediately under the clear ice cover at irregular intervals along the shoreline. It is within these air pockets that adults are presumed to have obtained surface oxygen, for live adults were subsequently collected on the surface as the ice melted in the early morning. To test for possible oviposition in the night, upper leaf debris and then 2–3 cm of underlying substrate were removed from the pond. Microscopic examination failed to reveal any eggs.

Such underwater activity was observed for 37 females during 4–18 April 1977. From 19 to 24 April, during late afternoon,

Female transference and mate choice among Tana River red colobus

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Red colobus are one of a small number of primate species in which females have been reported to transfer between breeding groups more commonly than males¹⁻³. Several authors have hypothesised that in such species transference may serve to reduce the risk to females of producing offspring of lower fitness through inbreeding¹⁻⁶. The hypothesis offers no explanation of why females rather than males are responsible for outbreeding in these species, but remains plausible so long as male membership of breeding groups is relatively stable; for once members of one sex have evolved dispersal mechanisms reducing the risk of inbreeding, pressures on the other sex to do likewise will be lowered⁵. Hence, if both sexes commonly migrate, the hypothesis is weakened. I describe here the membership dynamics of a group of Tana River red colobus, *Colobus badius rufomitratus*, which provide the first evidence of high rates of membership turnover by both sexes in primates, and speculate that the function of female transference in this case may be related to mate choice and the avoidance of infanticide.

The data come from a 3-yr study carried out between 1973 and 1975 near Wenje, in Eastern Kenya. The habitat consists of small patches of riverine forest, differing markedly in size, diversity and seasonality from the rain-forest habitats where red colobus are more widely distributed⁷. The social structure of the Tana population also differs from that reported from rain-forest sites in Tanzania and Uganda^{8,9}: instead of multimale groups of about 50 animals, the population is dispersed in groups of 12-30 animals, often containing only a single adult male and never more than two.

Reliable censuses of the main study group were obtained in 24 months between June 1973 and October 1975. During this period the size of the group varied from 16 to 33 animals, with a mean of 21.5. Only a single adult male was resident in the group at one time but there were two replacements during the study. The first occurred in July 1973 and was not observed, and the second took place 5 months later over a 3-day period in January 1974, after which the new male remained for at least another 21 months until the end of the study.

Changes in the number of adult females in the group are shown in Fig. 1. Net gains and losses seem not to have occurred in random sequence (runs test, $P < 0.05$ (ref. 10)), but rather to have involved a rapid decline, followed by a slow increase. The point of reversal in the trend coincides closely with the replacement of the second male in the group. When he joined in July 1973, the group contained 14 adult females and 4 clinging infants. By December, there remained only 8 females, 7 of which carried infants: at least 4 infants had been born in the interim, and one had died. Evidently, in this period pregnant females or those carrying infants tended to remain in the group, while those without young emigrated. On the other hand, after the second replacement in January, female numbers slowly increased to reach a maximum of 19 adults and 3 subadults by the end of the study. With only one exception the increase in adults was due to immigration rather than to the maturation of subadults within the group.

Evidence from known individuals on the origin of immigrants or the fate of emigrants is scarce. Of 11 individually recognisable females, 3 disappeared from the group and 2 were sighted elsewhere. One successfully transferred to a neighbouring group and remained there for at least 16 months, while the other was seen only once in a group some 2 km from the edge of the study

group's range. As solitary females were sighted only three times during the study, while solitary males were seen quite commonly, females probably transferred rapidly between groups.

These findings are of interest for three reasons. First, the rate of female movements was extremely high: a total of 34 were inferred over 29 months. Second, the migrations were made by adults 27 times and subadults 7 times, but never by independently-moving juveniles (although two moves were made by adult females carrying small juveniles). Among red colobus in Uganda, females also transfer between groups, but in this case juveniles are the mobile element and the migration of adults has not been reported³. If migration served simply to avoid inbreeding, transference at juvenile or subadult age would suffice, and the frequent migration of adults would not be expected. Third, the movements occurred despite two male replacements in the group. Again, if their function was to reduce the risk of inbreeding, they would seem to have been superfluous (unless, conceivably, successive males in the group were closely related individuals).

Alternative hypotheses to explain these findings are at present speculative. One possibility is that females shift between groups because the habitat is patchy and changeable, and thus provides an unpredictable food supply (for a similar hypothesis in birds, see ref. 11). If females transfer, however, because variation in the food supply in each group's range has a critical bearing on their fitness, then on finding a 'good' site, they should be expected to resist the entry of others. In this case the number of females present in the group more than doubled between December 1973 and July 1975, yet there was no evidence of serious aggression against newcomers. Moreover, at least 3 females entered during the months of June and July 1974, when food was apparently scarce⁷.

Another possibility is that females migrate because the males in harem groups remain for differing lengths of time, and their replacement brings a risk of infanticide, as may be the case in several other primate species¹²⁻¹⁵. During the 5 months after the third male joined the group, the number of infant deaths in relation to the numbers at risk was 3.1 times higher than the average over the whole study. Four out of seven infants present at the time of his entry died, and one emigrated with its mother. Another, born 2 weeks after the replacement, died within a week, after falling from a tree during or just before an unusual fight in which several females gave chase to the male.

In these circumstances, if females were able to assess the ability of a male to hold a harem during the time needed to conceive and rear an infant, they would benefit from choosing among neighbouring males before becoming pregnant and resident with one. The trait would then be expected to spread

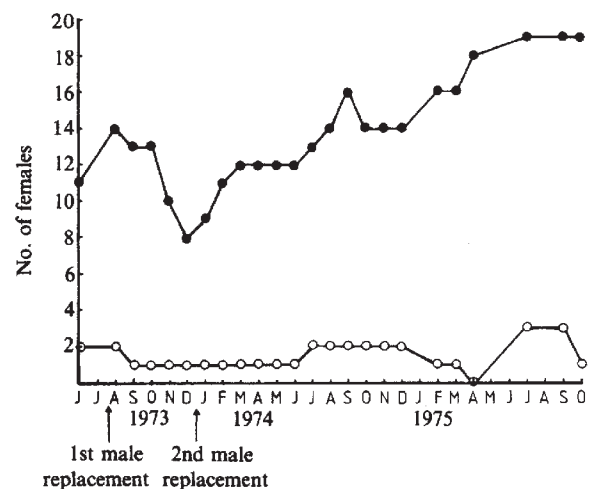


Fig. 1 Variation in the number of females in the Tana study group over 29 months. Also shown is the timing of two male replacements in the group. ●, Adult; ○, subadult.

rapidly in the population¹⁶. Such factors could account for the apparently greater preference by females for the third male in the study group, who was resident for at least 21 months, than for the second who remained for only 5 months. Further study of the problem would include a careful comparison of the behaviour of males to detect cues by which females might discriminate between them.

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Captopril (SQ 14225) depresses drinking and aldosterone in rats lacking vasopressin

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Angiotensin II is dipsogenic, and vasopressin (ADH) regulates renal water excretion. Together, these hormones govern overall mammalian water balance^{1,2}. The Brattleboro rat with inherited diabetes insipidus (DI) lacks ADH and is therefore a convenient model with which to elucidate mechanisms regulating water metabolism. In the present studies, angiotensin II has also been removed from DI rats by the administration of an inhibitor (captopril, SQ 14225; D-2-methyl-3-mercaptopropanoyl-L-proline) of the enzyme which converts angiotensin I, the relatively inert component of the renin-angiotensin system, to angiotensin II, the biologically active substance³. SQ 14225 reduced the drinking rates, and after 6 days lowered peripheral plasma aldosterone concentrations were associated with hyperkalaemia. We conclude that the polydipsia of diabetes insipidus partly results from elevated plasma renin activities and angiotensin II concentrations seen in this syndrome. Further, the apparent hypoaldosteronism of DI Brattleboro rats reflects differences in both tissue usage of the steroid and adrenocortical sensitivities associated with polyuria, hyperosmolality and possibly potassium wasting^{4,5}.

DI rats drink approximately eight times more water than normal animals^{6,7}, and in the present experiments each drank about 80 ml per 100 g body weight per 24 h during the initial 3-day control observation period. When the orally active SQ 14225 was introduced into the water (30 mg l⁻¹), the drinking rates were reduced by about 20% (Fig. 1). Control DI rats with a continuous supply of tap water drank at a consistent daily rate (76.4 ± 1.7 ml per 100 g per 24 h; n = 5) over 9 days. The drinking rate was not reduced because rats find SQ 14225 distasteful, as a daily stomach load of water containing the inhibitor lowered drinking rates to a similar extent (unpublished observations).

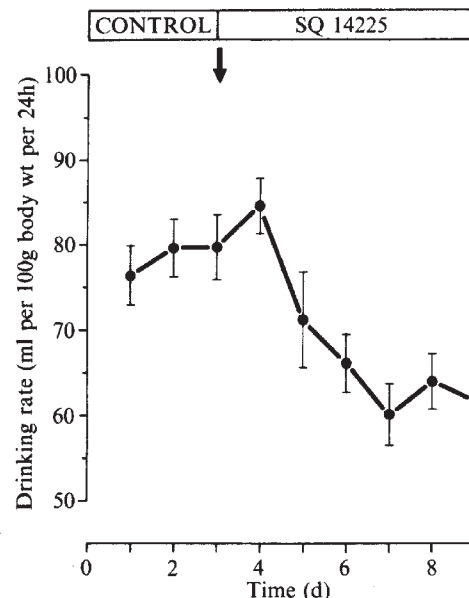


Fig. 1 The effects of SQ 14225 on drinking rate in male DI Brattleboro rats over a 9-day observation period. Rats were isolated for a 3-day adaptation period. Daily water consumption was measured for a further 3-day control period; after this, SQ 14225 was added to the drinking water (30 mg l⁻¹) at (↓), and drinking rates measured for a further 6 days, after which the animals were killed and blood collected. Each point is the mean ± s.e. of fluid intake of five rats. Values on days 7, 8 and 9 statistically different at $P < 0.05$. A control group of rats not given the drug at all, gave a drinking rate of 76.4 ± 1.7 ml per 100 g body weight per 24 h. Food intake and growth rates in control and SQ 14225-treated rats were similar.

Peripheral blood was collected from the abdominal aorta under ether anaesthesia on the morning of experimental day 10 (Fig. 1). Plasma osmolalities, sodium and corticosterone concentrations were similar in control and SQ 14225-treated rats (Table 1). Aldosterone concentrations were 66% lower in the SQ 14225-treated rats and plasma potassium concentrations slightly higher than control. Electrolytes were determined by flame photometry, osmolalities by freezing point depression and corticosteroids by radioimmunoassay⁸.

Inhibitors of enzymes responsible for the conversion of angiotensin I to angiotensin II have recently been used as potent antihypertensive drugs^{9–11}. In particular, those such as SQ 14225 reduced the blood pressure in renal hypertension as well as in hypertension which is free of overt abnormalities in the renin-angiotensin system. Their actions are highly complex, as angiotensin II, in addition to its dipsogenic actions, has feedback relationships with ADH¹², preferentially influences adrenocortical aldosterone biosynthesis, acts on the renal vasculature and has fairly specific roles in regulating blood pressure^{2,13}.

The severity of the polydipsia and polyuria of diabetes insipidus is attenuated by adrenocortical steroids; water intake and urine production rates fall by 50% after adrenalectomy of DI rats⁶. The present observed reduction in aldosterone concentrations without changes in corticosterone may indicate that the mineralocorticoid itself affects voluntary water intake either by direct actions on dipsogenic centres or indirectly as a result of alterations in sodium appetite and/or potassium balance¹⁴. The hypoaldosteronism, hypokalaemia and elevated plasma renin activity of DI rats may result from defective renal tubular mechanisms⁴, although a possibly increased metabolic clearance rate of aldosterone (R. J. Balment and I.W.H., unpublished observations) could effect similar changes. Indeed, the zona glomerulosa of DI rat adrenals is far more sensitive to angiotensin II than that of non-DI rats¹⁵.

Previous studies indicated that SQ 14225 increased drinking in DI Brattleboro rats¹⁶; in the same studies angiotensin, rather

Table 1 Effects of oral SQ 14225 on plasma electrolyte and corticosteroid concentrations in male Brattleboro rats with hypothalamic diabetes insipidus

	Control rats	Rats drinking SQ 14225 for 6 days
Aldosterone (ng %)	8.4 (5) ±2.0	2.8† (6) ±0.4
Corticosterone (µg %)	15.9 (5) ±2.8	12.4 (9) ±2.2
Potassium (mmol l ⁻¹)	3.82 (10) ±0.15	4.24* (9) ±0.21
Sodium (mmol l ⁻¹)	133.8 (5) ±3.2	141.6 (9) ±4.7
Osmolarity (mosmol l ⁻¹)	313.8 (5) ±6.0	307.8 (9) ±5.4

Results are means ±s.e. of numbers of rats given in parentheses.
* $P < 0.1$; † $P < 0.02$.

surprisingly, decreased drinking. Direct comparison with the present experiments is difficult, however, as the time course was short (2–6 h), whereas the present data suggest that at least 2 days of treatment are required for the depressed water intakes to become apparent, at least in the doses used. Other investigations have shown that SQ 14225 reduces peripheral plasma aldosterone concentrations in both normal and hypertensive patients¹⁷, whereas in deoxycorticosterone acetate-salt hypertensive rats drinking rates are reduced¹⁸.

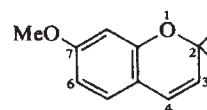
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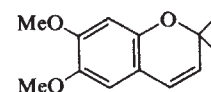
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production is suppressed, simulating removal of the glands. Active CA are required for the effect of P2 in *Oncopeltus*⁵. The CA are the site of monooxygenase (MO) activity associated with JH biosynthesis⁶ and in appropriate conditions, those isolated from certain adult insects can effect the terminal epoxidation of endogenous *E,E*-methylfarnesoate to give C₁₆JH (refs 7, 8). The destructive effect of P2 on the CA, together with the finding⁷ that the 3,4-dihydrodiol is a significant metabolite in some insects, suggested that P2 has a specific cytotoxic action within the glands, possibly mediated by local formation of the 3,4-epoxide¹⁰. Reactive epoxides of polycyclic aromatic hydrocarbons¹¹, bromobenzene¹², or aflatoxins¹³, and oxidative metabolites of other xenobiotics¹⁴ have been implicated in their cytotoxic, mutagenic and/or carcinogenic effects in mammals, in some cases with support from manipulations of the critical balance between activating (oxidising) and deactivating enzymes *in vivo*¹². We now present evidence that the action of precocenes on immature *Oncopeltus* and *Locusta* requires an oxidative bioactivation within the CA. The generation of reactive metabolites from innocuous precursors in vital tissue, if it can be linked to appropriate species and organ selectivity, offers an attractive approach to insect control.

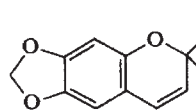
When early second instar *Oncopeltus* were exposed to deposits of various compounds and mixtures in glass jars, P2 at 1 µg cm⁻² gave total precocious metamorphosis at the 4th instar (Fig. 1), whereas 2H-P2 (compound V, below) had no effect at up to 5 µg cm⁻², above which mortality occurred. Thus, the 3,4-double bond is required for biological activity. The methylenedioxyphenyl (1,3-benzodioxole) analogue MDP(III) is also ineffective as an anti-allatin, despite its resemblance to P2 and similar polarity, as attested by an *R_F* value (TLC on silica gel; solvent, ethyl acetate/xylene, 25:75) lying in the range that includes P1, methylfarnesoate, P2 and C₁₆JH; a relationship which should ensure its reaching the cellular sites, especially those in the CA, that P1 and P2 reach.



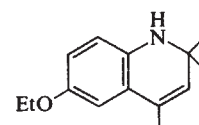
(I) P1*
(IV) 3,4-dihydro(2H-P1)
(VII) 6-methoxy-7H



(II) P2
(V) 3,4-dihydro-(2H-P2)



(III) MDP
(VI) 3,4-dihydro-(2H-MDP)



(VIII) Ethoxyquin

*Compounds except P2 were inactive when tested at 2 µg cm⁻² as in Fig. 1; P1 is active at 5 µg cm⁻² upwards.

If an oxidative bioactivation is required for precocenes' action, it is less likely to occur (self-stabilising effect) with MDP, which contains the 1,3-benzodioxole moiety, characteristic¹⁵ of certain insecticide synergists that act by inhibiting MO. Apart from any self-stabilising effect (believed to result in self-antagonism in this case) MDP should also inhibit MO that attack other molecules. Thus, in the cockroach CA bioassay of Pratt and Finney¹⁶, MDP inhibits (IC₅₀ ~ 3 × 10⁻⁶ M), more effectively than P2 (ref. 17), the epoxidation step in the conversion of exogenous farnesenic acid into C₁₆JH. These considerations make MDP an ideal candidate inhibitor of P2 oxidation in the CA *in vivo* and according to our hypothesis, a likely antagonist of its action. In fact, we find a clear-cut antagonism of P2 action by both MDP (Fig. 1) and its 3,4-dihydro-derivative (2H-MDP; VI) in *Oncopeltus*; normal moulting is restored at

The action of precocenes in milkweed bugs (*Oncopeltus fasciatus*) and locusts (*Locusta migratoria*)

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The active constituents of the plant *Ageratum houstonianum* causing early larval instars of some insects to moult into precocious prothellic adults are 7-methoxy-2,2-dimethylchromene and 6,7-dimethoxy-2,2-dimethylchromene¹—precocenes 1 and 2 (P1, P2). P2 causes atrophy of the corpora allata (CA) in at least two species²⁻⁵ and juvenile hormone (JH)

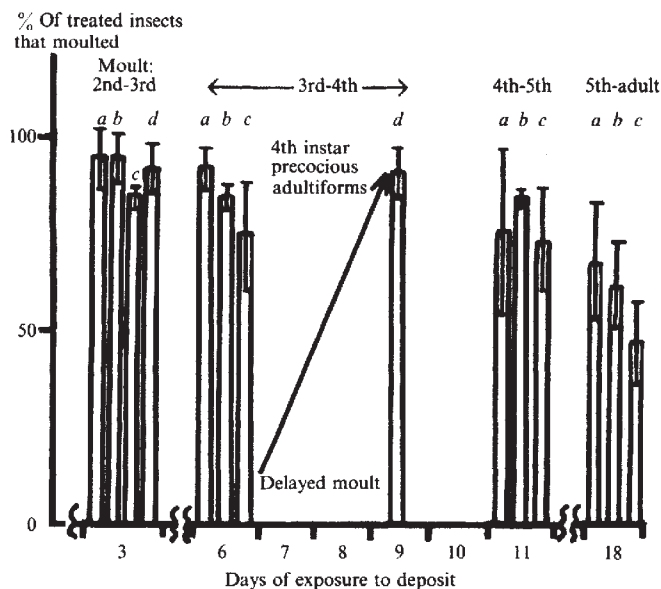


Fig. 1 Groups of 20 early 2nd instar *Oncopeltus* were confined (27°C; 2:1 light/dark regime) in 100 cm² glass jars (two groups per treatment) containing milkweed seed and water and surface treated with a, impregnating solvent (ether/acetone) only; b, MDP only (2 µg cm⁻²); c, MDP (2 µg cm⁻²) plus P2 (1 µg cm⁻²); or d, P2 only (1 µg cm⁻²). Except for P2 at the moult 3-4, values (mean of two experiments with range) represent insects in each exposure that moulted normally; others died. With P2 alone (d), the normal moult 2-3 is followed by delayed moulting (arrow) to either permanent or short-lived precocious 4th instar adultforms between days 6-9. When MDP (2 µg cm⁻²) is co-formulated with P2 (c), the normal 3-4 (day 6), 4-5 (day 11) and 5-adult moults are restored.

the 4th and 5th instar with MDP plus P2 (columns c in Fig. 1). In some experiments an increased number of deaths (compared with controls) as 5th instar larvae or during the adult moult, indicates a previously noted¹⁸ deleterious effect of P2 at the final moult which is probably unrelated to the anti-allatal effect. However, normal adults arising from antagonism tests with both MDP and 2H-MDP laid eggs that produced viable larvae. MDP showed no JH-activity towards *Oncopeltus* continuously exposed from the late 4th stage to 5 µg cm⁻² in the jar deposit

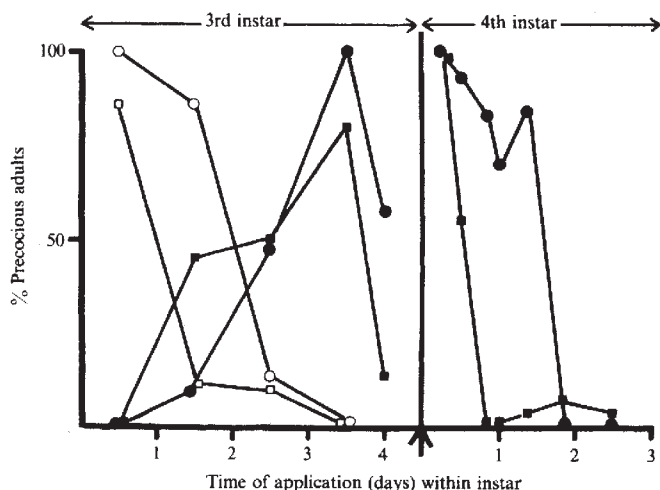


Fig. 2 Periods of sensitivity to the prothetelogenin action of precocene 1, during the 3rd and 4th instars of *Locusta*. We topically applied 50 µg (squares) or 100 µg (circles), in acetone (5 µl), to groups of 10 or 20 larvae at different times during development and recorded the percentage of animals exhibiting precocious metamorphosis at a subsequent ecdysis: hollow symbol, precocious 4th instars; solid symbols, precocious 5th instars. Solvent controls gave only normal animals.

test, or when topically applied (400 µg) in acetone to early 5th instar *Locusta*, or (40 µg) to newly moulted pupae of *Tenebrio molitor*.

Compounds such as 2,3-methylenedioxy-naphthalene and 2,4,5-trichloropropargyloxybenzene, recognised as 'insecticide synergist' type MO inhibitors¹⁵ gave indications of antagonism with P2 but closer analogues such as P1, 2H-P1 (IV), the P1-isomer(VII), 2H-P2 (V) and the antioxidant ethoxyquin which might inhibit P2 metabolism competitively, had no effect on its action. Thus, efficient antagonists must apparently bear a close structural resemblance to the precocenes and also interact strongly with the oxidase(s) concerned, in the manner of classical insecticide synergists. Sesamex, a JH-mimic for *Oncopeltus*¹⁹, had no apparent effect on development as a 2 µg cm⁻² deposit but showed partial antagonism in co-formulation with P2; neither peanut oil, liquid paraffin, tocopherol or tocopherol acetate protected against P2 at the 2:1 ratio, thereby eliminating the possibility of a diluent effect in jar deposits. Although sesamex might antagonise P2 action both by inhibiting MO and/or replacing JH, the antagonism is clearly incomplete in these experimental conditions, in contrast to that shown by MDP or 2H-MDP. Neither piperonyl butoxide nor tropital prevented precocious metamorphosis in these experiments.

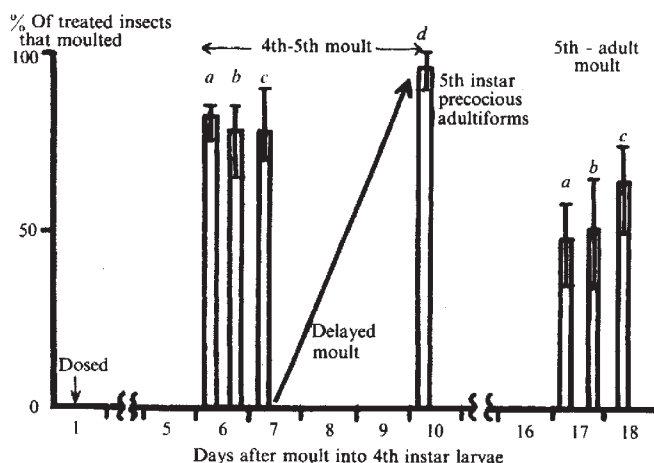


Fig. 3 Groups of 20 recently moulted 4th instar *Locusta* were topically treated with acetone (10 µl) alone, or containing 200 µg of MDP. After 1 h, insects were further treated with acetone (10 µl), alone, or containing P2 (100 µg), to give a, solvent control; b, MDP only; c, MDP/P2 (2:1); and d, P2 only test groups which were fed and held in a light/dark (2:1; 40°/25°C) regimen. Values (mean of three experiments with range) represent survival at each moult; precocious 5th instar larvae were not found in c, nor normal 5th instars in d. The slow moult (arrow) into permanent precocious 5th adultforms between days 7 and 10 with P2, is prevented by the combined treatment (columns c), which gives normal 5th instars and adults.

P1²⁰ and P2^{3,21} cause precocious metamorphosis in *Locusta* and we determined periods of optimal sensitivity to them, as in Fig. 2, which shows that treatment with P1 is effective throughout the 3rd and early in the 4th instar. Whereas P1 and P2 were fully effective at 100 µg when topically applied to early 4th instar larvae, neither 2H-P1 nor 2H-P2 caused precocious metamorphosis at doses up to 400 µg per insect, beyond which toxic effects appeared. As with *Oncopeltus*, the 3,4-double bond seems to be necessary for activity. MDP (200 µg) topically applied 1 h before either compound prevented the effect of 100 µg of P1 or P2. In Fig. 3, P2 applied alone delayed the moult from 4th to 5th instar and produced permanent precocious 5th adultforms (column d), whereas normal 5th instar larvae and adults resulted from the MDP/P2 combination (columns c).

Groups of five 4th instar *Locusta*, treated with P2 or MDP/P2 as in Fig. 3, were surface rinsed with ether/acetone (4:1) and the

carcasses extracted with this solvent, 3 h after application of P2. Quantitation by GLC showed that up to three times more P2 remained in the insect tissues when MDP was also present, demonstrating that this 1,3-benzodioxole analogue indeed inhibits the oxidative metabolism of P2. MDP can evidently maintain its protective effect within the CA, despite the possibility that more unmetabolised P2 arrives there because of the inhibition of its metabolism in peripheral tissues. Similar effects were observed in studies on the pharmacokinetics of 4-ipomeanol, a toxin that acts on mammalian lung by oxidative metabolism²².

Earlier work¹⁷ showed that P2 progressively inhibited the final steps, including epoxidation, of C₁₆JH formation from farnesenic acid in the CA of the cockroach, *Periplaneta americana*, *in vitro*. Further experiments are in progress to test our hypothesis that labile precocene 3,4-epoxides, or derivatives thereof, are proximate insect- and cell-specific cytotoxins (alkylating agents) whose formation in competition with the epoxidation step of JH biosynthesis in the CA of sensitive insects destroys the glands' ability to synthesise the hormone.

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Cell size changes in undeprived laminae of monkey lateral geniculate nucleus after monocular closure

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Transneuronal degeneration in the lateral geniculate nucleus (LGN) due to removal of an eye or closure of the eyelids has traditionally been measured by comparing the sizes of cells in the deprived laminae with those of the corresponding undeprived laminae in the nucleus of the opposite side of the same brain. However, the undeprived laminae may themselves change; some hypertrophy of their cells has been found in kittens following early monocular closure¹ but such comparisons are difficult in the cat because of the variation between animals². In a recent study of the effect of binocular closure in the monkey³ it was necessary to measure cell sizes in the LGNs of a series of normal monkeys as controls, because there are no undeprived laminae after binocular closure. From this series of normal animals it was apparent that there is much less variation between individual monkeys than has been found with cats and it was thus possible to make reliable comparison of cell sizes

between different animals. In the present study the series of normal monkeys has been extended and the mean cell size and its variability between animals have been established for each geniculate lamina in the normal rhesus monkey. We give here a comparison of the cell sizes of both deprived and undeprived laminae following monocular eyelid closure with normal measurements and report some unexpected findings, particularly in animals where monocular closure has been carried out relatively late.

Eight normal monkeys formed the control group, five which were perfused at 8, 9, 17, 32 and 32 days of age and three young adults. In six more animals the lids over one eye were sutured under open ether anaesthesia at various ages and the young monkeys returned to the mother for various periods (Fig. 1). As both these variables have been found to be important the results have been analysed in two groups: early closure, long survival (2) and late closure (4). Details of the methods of fixation, histological preparation and measurement have been described previously³. Fifty cells were measured in each lamina of each animal and the sample was taken from the middle of the lateral limb at the junction of the middle and posterior thirds of the nucleus; only cells with distinct nucleoli were drawn. To make comparisons between animals the mean cell area for each lamina in each animal has been treated as a single observation and the averages of all the means and standard deviations of these values have been calculated from these for each group (Tables 1-3).

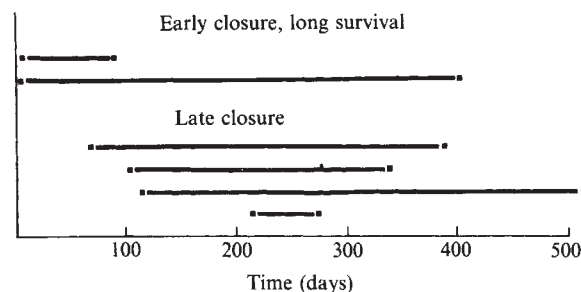


Fig. 1 The age at monocular closure and duration of closure for animals in this study.

Comparisons between groups have then been made for each lamina using Student's *t*-test. Values from the left and right sides of control animals have been treated separately throughout as have the deprived and undeprived laminae of experimental animals. In addition, for each lamina of the normal animals, the range within which 95% of the mean cell areas for a single normal lamina would be expected to fall has been calculated so that individual experimental animals may also be compared with the normal group.

Analysis of the cell area measurements for each lamina in the eight normal animals shows the degree of variability between animals; individual details of five have been published³, and the small variability of the means from each animal about the group means can be seen from the small standard deviations of the normal measurements (Table 1). The variability in the parvocellular laminae (III to VI) is considerably less than in the magnocellular laminae (I and II). Comparison of the five infants with the three adults shows little or no growth occurring in the

Table 1 Mean LGN cell areas of normal animals (μm^2)

Lamina	Overall <i>n</i> = 16† s.d.	Infant <i>n</i> = 10† s.d.	Adult <i>n</i> = 6† s.d.
I	298 ± 26	291 ± 28	309 ± 21 NS
II	294 ± 35	277 ± 30	322 ± 21*
III	196 ± 14	200 ± 12	189 ± 17 NS
IV	195 ± 9	198 ± 10	191 ± 8 NS
V	191 ± 13	191 ± 15	192 ± 8 NS
VI	183 ± 11	180 ± 12	190 ± 7 NS

Significances shown against the adult measurements are for differences from the infant group.

NS, not significant. *, *P* > 0.05, †, Two sides from each animal.

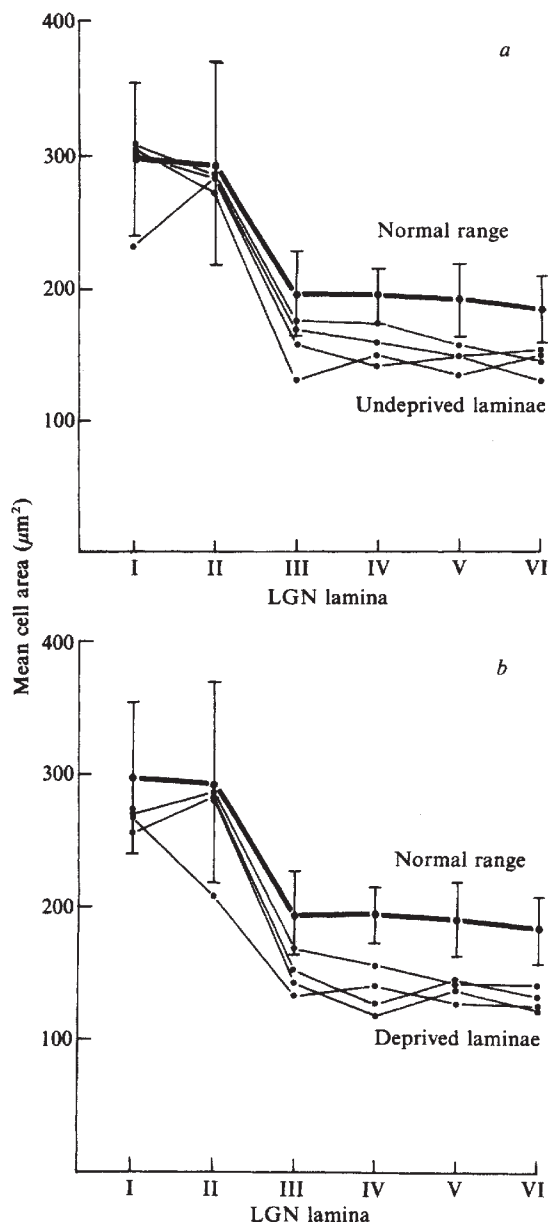


Fig. 2 Mean areas of neuronal somata in the undeprived LGN laminae (a) and deprived laminae (b) of four monkeys following late monocular closure. Lines join the measurements for an individual animal. Each bar for the normal range indicates an interval within which the mean cell area in a single lamina from one animal may be expected to lie with a probability of 0.95. Most of the values for both the deprived and undeprived parvocellular laminae fall below the normal range indicating shrinkage of cells in these laminae.

parvocellular laminae within the ages studied but there is a little growth in the magnocellular laminae. Measurements of all eight normal animals have therefore been pooled to determine the mean and variability of each lamina in the normal monkey. This variability is much less than that of 'normal' laminae in monocularly deprived animals.

The measurements in the four monkeys with late monocular closures show little shrinkage when the deprived laminae are compared with the undeprived laminae (Table 2). This would be interpreted as showing the decline in sensitivity towards the end of the critical period. However, if these measurements are compared lamina by lamina with those of the normal animals, there is a 20–25% shrinkage of the undeprived parvocellular laminae and this is masking a shrinkage of ~30% in the deprived parvocellular laminae (Table 2, Fig. 2). The undeprived magnocellular laminae do not show a comparable effect and the

Table 2 Mean LGN cell areas from late closures (μm^2)

Lamina	Undeprived	% Change from normal	Deprived	% Change from normal	% Difference between undeprived and deprived
	s.d.		s.d.		
I	286 ± 36	↓4 NS	268 ± 9	↓10*	↓6 NS
II	281 ± 7	↓4 NS	265 ± 38	↓10 NS	↓6 NS
III	157 ± 19	↓20*	150 ± 15	↓23†	↓4 NS
IV	156 ± 14	↓20†	135 ± 17	↓31†	↓13 NS
V	146 ± 9	↓24†	138 ± 8	↓28†	↓5 NS
VI	143 ± 10	↓22†	131 ± 8	↓28†	↓8 NS

NS, not significant.

* $P < 0.01$; † $P < 0.001$; $n = 4$.

shrinkage of the deprived magnocellular laminae is much less than that of the deprived parvocellular laminae. The differences between deprived and undeprived cell sizes after monocular eyelid closure are thus very similar for the magnocellular and parvocellular laminae. Late monocular closure thus causes a marked shrinkage of the parvocellular laminae but it affects both deprived and undeprived laminae to almost the same extent.

Table 3 shows the mean measurements for the two monkeys with early closure and long survival. Comparison of deprived with undeprived laminae shows shrinkages of 20–30%, in agreement with previous reports. However, if the measurements are compared with those of the normal animals it is apparent that there is a consistent shrinkage of ~10% in the undeprived parvocellular laminae. This means that the shrinkage in the deprived laminae is underestimated by this amount in the traditional method.

As the undeprived magnocellular laminae show little change they form an internal control for the changes in the parvocellular laminae. This may be measured by calculating the ratio of the mean cell area of the magnocellular laminae to that of the parvocellular laminae, taking deprived and undeprived laminae separately. Table 4 shows these ratios for each animal and that the ratio is consistently higher following monocular closure for both deprived and undeprived laminae. In any one animal the ratio changes for deprived and undeprived laminae are remarkably similar, although the mean cell areas from which they are calculated may differ markedly; this indicates that the excess shrinkage of the deprived parvocellular laminae compared with the deprived magnocellular laminae is closely similar to the shrinkage of the undeprived parvocellular laminae compared with the undeprived magnocellular laminae. As well as the generally accepted shrinkage of the deprived laminae in relation to the undeprived laminae following late monocular closure there is thus an additional and larger shrinkage affecting both deprived and undeprived parvocellular laminae equally. The early closure, long survival group shows a similar but smaller shrinkage of the undeprived parvocellular laminae (although the survival periods were comparable in the two groups), indicating

Table 3 Mean LGN cell area of early closure—long survival (μm^2)

Lamina	Undeprived	% Change from normal	Deprived	% Change from normal	% Difference between undeprived and deprived§
	s.d.		s.d.		
I	328 ± 21	↑10 NS	243 ± 24	↓18†	↓26‡
II	313 ± 10	↑6 NS	242 ± 20	↓18†	↓23‡
III	179 ± 2	↓9†	128 ± 12	↓35‡	↓28‡
IV	176 ± 17	↓10 NS	131 ± 11	↓33‡	↓26‡
V	172 ± 10	↓10*	122 ± 1	↓36‡	↓29‡
VI	162 ± 8	↓11†	111 ± 19	↓39‡	↓31‡

NS, not significant; *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$. §, Significance levels in this column are based on the conventional analysis using a comparison of sides within each animal and the standard errors of the 50 cells in each sample are used. Both animals separately achieved the significance level shown for each lamina. $n = 2$.

that this effect increases with age at monocular closure within the age limits of the study.

We suggest, therefore, that the development of the primate visual system occurs in two major phases. Initially the anatomical balance between the connections of the two eyes is established, corresponding to the critical period as presently defined and the period of plasticity of the cortical ocular dominance columns^{4,5}; it is during this period that monocular closure may produce hypertrophy¹. As these connections become established we suggest that the presence of synchronous inputs from the two eyes becomes necessary for the establishment of normal binocularity in the cortex. In the absence of corresponding visual inputs it is no longer possible for both eyes to drive the same cell effectively simultaneously and so the connections from the two eyes interfere with each other and the development of both is inhibited. This interference thus differs from the competition described in the cat during the critical period⁶.

Table 4 Ratios of magnocellular to parvocellular cell areas

Normals	Age (days)	Ratio
OM 216	8	1.65
OM 222	9	1.45
OM 225	17	1.26
OM 169	32	1.46
OM 221	32	1.61
OM 185	Adult	1.71
OM 187	Adult	1.68
OM 206	Adult	1.60
	Mean	1.55
Early monocular closure, long survival		
	Normal	Ratio
OM 166	1.82	1.85
OM 210	1.90	2.12
	Mean 1.86*	1.99*
Late monocular closure		
OM 200	1.79	1.81
OM 193	2.04	2.12
OM 212	1.83	1.83
OM 194	1.87	1.94
	Mean 1.88*	1.93*

Significances shown are for differences from the group of normal animals.

* = $P < 0.001$.

Three major conclusions may be drawn by comparing the results of monocular closure with those of normal monkeys. First, following late monocular closure or early closure and a long survival period there is shrinkage of the undeprived parvocellular laminae; this leads to underestimation of the degree of shrinkage of the deprived parvocellular laminae if the undeprived laminae are used as a control. The primate visual system is thus more sensitive to monocular closure in older animals than had previously been supposed, with increasing effect on the undeprived laminae. Second, as this effect is restricted to the parvocellular laminae which represent the X-cell system while the magnocellular laminae represent the Y-cell system, these two functional pathways react differently to monocular deprivation. Third, as the cell areas measured in the late closure group for all the deprived laminae and the undeprived parvocellular laminae are smaller than those in normal animals at the corresponding ages of closure it can be concluded in this group at least that actual shrinkage of cells has taken place rather than failure of growth.

Axonal transport of vasoactive intestinal peptide in sciatic nerve

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Dense plexuses of neurones containing immunoreactive vasoactive intestinal peptide (VIP) have been found in discrete areas of the central nervous system and in peripheral organs, including the gastrointestinal tract, pancreas and urogenital system¹⁻⁷. In many of these locations VIP is concentrated in nerve endings, where it can be released by high K^+ concentrations in a Ca^{2+} -dependent manner^{8,9}. VIP release may also be provoked by electrical stimulation of nerves, for example the vagus¹⁰. VIP thus shows some of the features of neurotransmitter or neuromodulator substances. The presence of immunoreactive VIP in the fine terminal varicosities as well as in the cell bodies of neurones^{4,11} suggests that it might be transported from the perikaryon, where it is presumably formed, to the nerve endings, through the axonal transport system¹²⁻¹⁴. Such transport would be in keeping with a role for the peptide as a neurohumor or neurohormone¹⁵. We report here that VIP accumulates in constricted rat sciatic nerves in a manner suggesting fast, anterograde axonal flow.

Under light ether anaesthesia, the left sciatic nerve in male Sprague-Dawley rats was constricted by tying a silk thread around the nerve below the hip. The contralateral nerve was isolated but not ligated. After 4, 12, 24 or 72 h, the rats were killed by decapitation. The dissected sciatic nerves were cut in consecutive segments (0.5 cm each), beginning with the segment immediately proximal to the ligature. Two or three identical segments from different experiments were pooled, homogenised in 1 ml of ice-cold acetic acid (0.2 M); the homogenates were centrifuged at 8,000 g for 20 min and the supernatants were analysed for immunoreactive VIP by a sensitive and specific radioimmunoassay¹⁶. In four further experiments, the left sciatic nerve was ligated at two points, 1 cm apart; the segment between the two ligatures as well as an equal segment distal to

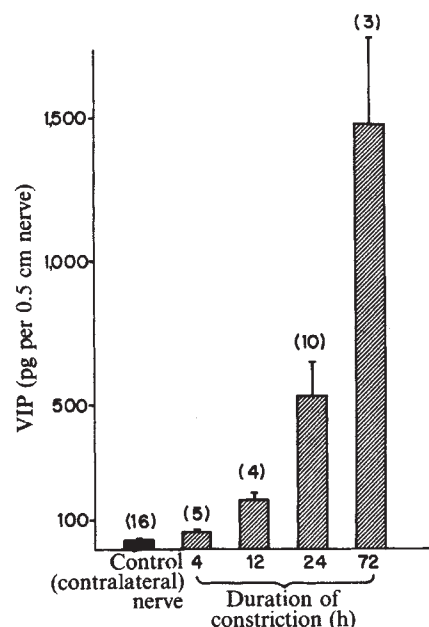


Fig. 1 Accumulation of VIP in proximal segment of sciatic nerve constricted for 4, 12, 24 or 72 h. Values are means $\pm$ s.e.m. Number of determinations in parentheses.

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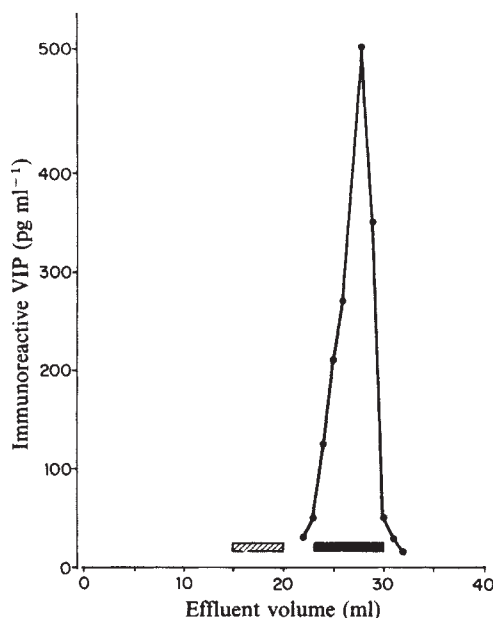


Fig. 2 Chromatogram of VIP immunoreactivity in an extract of sciatic nerves (402 mg) on a column of Sephadex G-25 Fine (0.9×60 cm). The nerves had been homogenised in 25 ml 0.2 M acetic acid, the homogenate centrifuged, and the supernate lyophilised and reconstituted in 0.5 ml of 0.2 M acetic acid. The column was calibrated with ^{125}I -labelled porcine VIP (solid bar) and ^{125}I -labelled albumin (shaded bar). Eluant, 0.2 M acetic acid; flow rate, 12 ml h^{-1} ; fraction vol., 1 ml.

the second ligature were homogenised and assayed for VIP content.

In the control (unligated) nerve, VIP content was uniform in all segments examined and averaged 32 ± 5 pg per segment. At all intervals after nerve constriction, the concentration of immunoreactive VIP increased significantly ($P < 0.025$) in the segment just proximal to the ligature. The peptide levels increased with the duration of constriction (Fig. 1), reaching 17 and 46 times the control value after 24 and 72 h, respectively. Accumulation of the peptide occurred only in the segments proximal to the constriction (Table 1), and peptide levels decreased abruptly above the first segment (0–0.5 cm). VIP content also remained low in the segment below a second constriction (14 pg), and in the segment between two ligatures (24 pg).

On chromatography of extracts of sciatic nerves on a column of Sephadex G-25 Fine, immunoreactive VIP was eluted in one peak. The location of this peak was identical for ligated (12–72 h) and unligated nerves, and coincided with the peak of ^{125}I -labelled VIP chromatographed in the same conditions (Fig. 2). These chromatographic data suggest the presence of one molecular species of the peptide in ligated or unligated sciatic nerves.

The findings indicate axonal transport of VIP along the sciatic nerve predominantly in a proximal-to-distal direction. The absence of a concentration gradient of VIP along the contralateral nerve or in the constricted nerve more proximal to the

segment immediately above the ligature suggests that the peptide is not formed *in loco*, but is probably synthesised in neuronal cell bodies and transported along the axon toward the nerve endings.

The rate of axonal transport of VIP (in mm h^{-1}) could be computed as: $(C - C_c/T)/(C_c/S)$, where C and C_c are the concentrations of the peptide above a constriction and in the contralateral nerve, respectively, T is the duration of constriction (in h), and S is the length of the nerve segment (in mm). From the data after 24 or 72 h constriction, the velocity of axonal transport of VIP was 3.3 mm h^{-1} . This velocity is typical of the relatively fast axoplasmic transport rates reported for cholinesterase in frog sciatic nerve¹⁷, for certain polypeptides synthesised in rabbit retinal ganglion cells¹⁸ and for other systems¹⁹.

Axonal transport of peptides has previously been documented for oxytocin and vasopressin in the hypothalamo-neurohypophyseal system^{20–22}. Our results now show that neuronal peptides can travel by axonal transport in peripheral nerves as well. The demonstration of axoplasmic flow of VIP supports the notion that this peptide may function as a neurotransmitter or neuromodulator in peripheral nerves.

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GABA of CNS origin in the rat anterior pituitary inhibits prolactin secretion

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Table 1 Concentration of immunoreactive VIP in 0.5-cm segments of sciatic nerve proximal to constriction for 24 h

Segment	VIP (pg per nerve segment)
0–0.5	527 ± 117 (10)
0.5–1.0	65 ± 10 (10)
1.0–1.5	102 ± 8 (5)
1.5–2.0	103 ± 6 (5)

VIP values are means $\pm$ s.e.m. Number of determinations are in parentheses.

Hypothalamic hormones synthesised in nuclei within the medial basal hypothalamus (MBH) and extrahypothalamic regions and released from nerve endings at the level of the median eminence (ME) into the hypophyseal portal capillary system control anterior pituitary (AP) function¹. The median eminence is a key area for neuroendocrine regulation as it receives projections of neurotransmitter pathways which modulate functions such as

synthesis and release of hypothalamic hormones². In addition, transmitters released from the ME into the stalk portal blood may act directly at the AP level to control hormone secretion—this has been demonstrated for dopamine (DA) with respect to prolactin (PRL) secretion³ and it has been suggested that DA itself may represent the so far elusive PRL-inhibiting factor (PIF)^{2,4,5}. Recently, evidence has been presented which suggests that γ -aminobutyric acid (GABA) may also have an important inhibitory role in regulating PRL secretion in the rat. Both GABA and muscimol, a powerful agonist at GABA receptors⁶, inhibited PRL secretion when injected into urethane anaesthetised female⁷ or freely moving male rats⁸. In view of the poor penetrability of the blood-brain barrier (BBB) by both amino acids⁹ and the recently described specific GABA receptor sites in rat AP¹⁰, the above data support the notion that GABA may act directly at the level of the AP to inhibit PRL secretion. This proposition is reinforced by the demonstration that both GABA¹¹ and muscimol¹² inhibit PRL secretion from rat isolated APs, an effect which is blocked by the antagonist picrotoxin. We report here that GABA, derived from the central nervous system (CNS), is present in the rat anterior pituitary, and we provide evidence that GABA has a functional role in the control of PRL secretion.

Male Sprague-Dawley rats (150–200 g) were killed by microwave radiation and their GABA content in the anterior and posterior pituitary lobes was determined by a mass-fragmentographic technique¹³ and compared with DA titres in the same tissues.

Table 1 shows that GABA is present in both the anterior and posterior lobes of the pituitary, the concentration of the amino acid being 7–8 times greater in the latter than the former tissue. DA shows a similar pattern of distribution in the pituitary, although absolute levels of the amine in both tissues are consistently lower than those of the amino acid.

Endogenous GABA in the brain is synthesised almost exclusively by decarboxylation of glutamic acid by glutamic acid decarboxylase (GAD)¹⁴ and GABA is degraded by a transamination catalysed by α -ketoglutarate-GABA transaminase (GABA-T)¹⁵. It is evident from Table 1 that the AP, despite the presence of GABA, does not contain the GABA biosynthetic machinery; no GAD activity was detectable at this level whereas GAD activity was present in the hypothalamus and, although at low levels, also in the posterior lobe. This finding is analogous to the finding that DA is present in the AP even though its biosynthetic enzyme, tyrosine hydroxylase cannot be detected¹⁶.

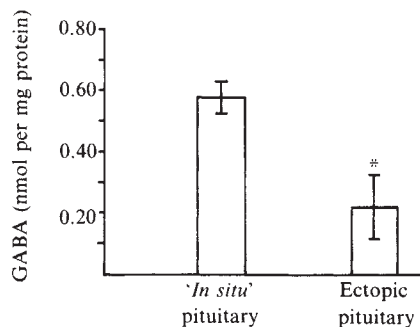


Fig. 1 GABA concentration in the ectopic or *in situ* anterior pituitary gland. Female Sprague-Dawley rats (100–130 g) were hypophysectomised by the transauricular route³⁶ and 15 days later received implants of one anterior pituitary, obtained from an intact female littermate, under the kidney capsule. After transplantation and before the experiments, animals were weighed daily; only animals which showed a constant body weight increase throughout this period were included in these studies. GABA concentrations were determined in the ectopic gland 10 days after transplantation (ref. 13). Age- and weight-matched female rats served as controls. Significance of differences between groups was calculated by Student's *t*-test. Values represent the mean $\pm$ s.e.m. of six determinations. **P* < 0.01.

a further similarity between GABA and DA at AP level is the fact that for both the amino acid transmitter and the amine, the physiological catabolic enzymes, that is, GABA-T (Table 1) and monoamine oxidase¹⁶ respectively, are present in the glandular tissue. GABA-T activity was also detected in the hypothalamus at concentrations similar to those found in the AP (Table 1).

Taken together these findings argue against the possibility of local formation of GABA in the AP and suggest instead that the gland may merely be a tissue for storage and catabolism of GABA manufactured elsewhere.

Ethanolamine-*O*-sulphate (EOS), a structurally related analogue of GABA, is a potent, specific, competitive and irreversible catalytic inhibitor of GABA-T *in vitro* and *in vivo*¹⁷. Experiments were performed to determine whether GABA stored in the AP originated in the CNS. Table 2 shows that EOS injected intracerebroventricularly (i.v.t.) at 2 mg per kg body-weight into the lateral ventricle of freely behaving unanaesthetised rats induced an increase in GABA content not only in the hypothalamus but also in the anterior and posterior pituitary

Table 1 GABA concentrations and GAD and GABA-T activities in rat pituitary lobes and hypothalamus

Tissue	GABA (nmol per mg protein)	GAD (pmol CO ₂ per mg protein per h)	GABA-T (nmol glutamate per mg protein per h)	Dopamine (pmol per mg protein)
Anterior lobe	0.76 $\pm$ 0.07	None	324.6 $\pm$ 21.1	1.25 $\pm$ 0.13
Posterior lobe	5.53 $\pm$ 0.60	0.574 $\pm$ 0.017	245.2 $\pm$ 21.2	53.71 $\pm$ 10.01
Hypothalamus	40.11 $\pm$ 3.81	6,320 $\pm$ 450	224.7 $\pm$ 17.0	66.63 $\pm$ 3.22

To avoid variability due to circadian fluctuations of GABA content in the hypothalamus²⁸, these and the following experiments (Tables 2, 3 and Fig. 1) were conducted in the early afternoon. The brain and pituitaries from microwave-irradiated (2.0 kW; 2.45 GHz; 75 W cm⁻²)²⁹ male Sprague-Dawley rats were rapidly removed, and the hypothalamus was dissected from the brain according to the procedure described in ref. 30. The weight of an hypothalamic fragment was about 20–25 mg. Pituitaries once removed were washed in cold saline and posterior and intermediate lobes were entirely removed from the isthmus to assure separate analysis of pure anterior and posterior (neural and intermediate) lobes. The mean weight of an anterior or posterior pituitary expressed as mg protein was about 0.5 or 0.1, respectively. For GABA determination, excised hypothalami and pituitary lobes were immediately frozen in dry ice, and then homogenised in 0.1 M formic acid (1 ml for hypothalamus and 200 μ l for pituitary lobes) containing the internal standard 4-aminovaleric acid (2 μ mol ml⁻¹). The homogenates were centrifuged at 15,000g for 20 min and an aliquot of the supernatant was transferred and evaporated under reduced pressure. A mixture of SIL PREP and BSTFA (3:1) (Applied Science, Lab. Inc. State College, Bellefonte, Pen.) was added. After incubation for 2 h at room temperature, an aliquot of the reaction mixture was injected into a gas chromatograph-mass spectrometer (Finnigan 3000 E), focused on *m/e* 304 and 174 for GABA-TMS derivative and *m/e* 304 for the internal standard according to Cattabeni *et al.*¹³. DA was also analysed with a mass fragmentographic technique, as described by Cattabeni *et al.*³¹. GAD was assayed by measuring the evolution of ¹⁴CO₂ from L[1-¹⁴C]glutamic acid in anaerobic conditions³². The ¹⁴CO₂ formed was absorbed in hyamine solution and counted in a liquid scintillation counter. In a typical assay, the incubation vessel contained 0.1 ml of 0.208 M glutamic acid (0.74 μ Ci L[1-¹⁴C]glutamate) in 0.1 M K₃PO₄ buffer containing 0.2 mM pyridoxal phosphate (PLP) pH 7.2. The reaction was started by injecting 1 ml enzyme solution in 50 mM K₃PO₄ buffer, pH 7.2, containing 0.2 mM PP and 1 mM AET (standard buffer) into the incubation vessel. The incubation was performed in a Dubnoff metabolic incubator for 30 min at 37 °C at approximately 150 r.p.m. and was terminated by injecting 0.1 ml of 4 M H₂SO₄ into the reaction mixture. To ensure complete release of CO₂ and absorption in the hyamine base, vessels were incubated for additional 60 min. GABA-T activity was assayed according to Macaione³³ in 100 μ l of the reaction mixture containing: GABA, 5 μ M; α -ketoglutarate, 5 μ M; PLP, 0.4 μ M; 0.1 M phosphate buffer, pH 7.4 and homogenate (w/v:1:10). After 1 h incubation at 37 °C, the reaction was stopped by adding 0.4 ml of absolute ethanol. Controls were carried out by adding homogenate after ethanol. GABA content in the homogenate and glutamic acid formed by GABA-T activity were quantitated by means of an amino acid analyser (Carlo Erba). Proteins were determined by the procedure of Lowry *et al.*³⁴. For GABA and DA, values represent the mean $\pm$ s.e.m. of at least five determinations; for GAD in the pituitary lobes and hypothalamus, values represent the mean $\pm$ s.e.m. of five determinations obtained from a pool of 10 glands or 4 hypothalamic fragments, respectively; for GABA-T in the AP and hypothalamus, values represent the mean $\pm$ s.e.m. of 6 determinations obtained from 12 tissue samples.

lobes. In the AP the increase in GABA concentration was time related. EOS does not cross the BBB¹⁷ so it is unlikely that these changes are caused by an effect of the drug on GABA catabolism in the systemic circulation. Supporting this view is the observation that intravenous injection of EOS, at a dose 25 times greater than the i.v.t.-injected dose, that is, 50 mg per kg, did not alter GABA concentrations either in the hypothalamus or the pituitary gland (Table 2).

If, as implied by experiments with EOS, the main source of GABA in the AP is via transport of the amino acid from the CNS to the pituitary, location of the pituitary in a site far removed from the sella turcica should result in a lowering of the pituitary concentration of the amino acid. Figure 1 shows that in a gland transplanted under the kidney capsule, the GABA concentration is three times lower than in the pituitary gland *in situ*.

Table 2 GABA levels in rat pituitary gland and hypothalamus after central or systemic administration of ethanolamine-*O*-sulphate (EOS)

Treatment	GABA (nmol per mg protein)		
	Anterior lobe	Posterior lobe	Hypothalamus
Central route			
Saline 10 µl per rat			
	0.64 ± 0.07	6.05 ± 1.26	48.21 ± 5.40
EOS (2 h)	1.27 ± 0.35*	13.18 ± 3.31*	120.65 ± 15.61*
EOS (4 h)	3.17 ± 0.87*†	19.32 ± 4.97*	150.41 ± 24.50*
Systemic route			
Saline 0.5 ml per rat			
	0.71 ± 0.18	4.28 ± 1.10	40.11 ± 3.81
EOS (2 h)	0.59 ± 0.20	4.32 ± 1.67	41.33 ± 4.03
EOS (4 h)	0.60 ± 0.17	5.47 ± 1.91	39.67 ± 3.63

In the central experiments, male Sprague-Dawley rats (200–220 g) had a cannula implanted into the lateral ventricle of the brain³⁵ while under barbiturate anaesthesia. After allowing 2–3 days for recovery, EOS (2 mg per kg in 10 µl saline) was slowly infused through the implanted cannula. When EOS was injected by systemic route, an intravenous (i.v.) dose of 50 mg per kg was used. Animals were killed by microwave radiations 2 and 4 h after EOS administration. Tissues were dissected and assayed for GABA as described in Table 1 legend (ref. 13). Values represent the mean ± s.e.m. of 10 determinations (pooled data of two separate experiments). Significance of difference between groups was calculated by Student's *t*-test.

**P* < 0.01 versus saline-injected rats;

†*P* < 0.01 versus EOS-injected rats (2 h).

Proof that GABA, probably derived from the CNS and transported into stalk blood to the AP, exerts a functional role in the control of PRL secretion is provided by the experiments summarised in Tables 3 and 4. Table 3 shows that, concomitant with an increase in hypothalamic content of GABA by i.v.t.-injected EOS, there was a clear-cut decrease in plasma concentration of PRL; in contrast, EOS administered systemically affected neither hypothalamic GABA concentrations nor plasma PRL titres. The case for a causal relationship between changes induced in the hypothalamic and pituitary concentrations of GABA and changes in PRL release is strengthened by the observation that they occurred independently of alterations in DA function. In fact, i.v.t. administration of 2 mg per kg EOS, which induced clear-cut rises in GABA concentrations in the hypothalamus and the pituitary, did not affect the DA level in the AP, which is a reliable marker of tubero-infundibular DA function¹⁸ (2.5 ± 1.03 and 2.9 ± 0.35 pmol per mg protein in saline- and EOS-injected rats, respectively). Furthermore, central administration of EOS antagonised the PRL-releasing effect evoked by blockade of DA receptors with domperidone, a drug which, being unable to cross the BBB^{19,20}, acts almost exclusively at the AP level (Table 4).

Although GABA is a neurotransmitter involved in the control of AP hormone secretion², the mechanism(s) underlying its neuroendocrine effects have yet to be clearly established. For instance conflicting data have been presented, indicating both a stimulatory^{21–23} and, an inhibitory^{7,8,12,24} role for GABA in the

regulation of PRL secretion. The data presented here show GABA to be present in the AP gland and offer a plausible explanation for the mechanism underlying the PRL-lowering effect of the amino acid.

Table 3 Prolactin concentrations in the plasma and GABA content in the hypothalamus after i.v.t. or i.v. administration of EOS

Treatment	Prolactin (ng ml ⁻¹)		GABA (nmol per mg protein)	
	EOS (2 mg per kg) central route	EOS (50 mg per kg) systemic route	EOS (2 mg per kg) central route	EOS (50 mg per kg) systemic route
Saline	20.5 ± 5.5	14.6 ± 3.5	40.01 ± 2.13	45.50 ± 2.60
EOS (2 h)	3.0 ± 0.9*	17.1 ± 1.9	138.99 ± 13.60*	40.20 ± 4.09
EOS (4 h)	9.5 ± 4.2*	15.4 ± 2.4	161.18 ± 24.11*	38.90 ± 3.90

In these experiments central and systemic administration of EOS were performed following the schedule described in the legend to Table 2. Immediately before and 2 and 4 h after EOS administration, male rats were sampled by the retroorbital venous technique³⁷ for PRL determination in the plasma. PRL was determined by a double antibody radioimmunoassay (RIA), using the method of Niswender *et al.*³⁸. All results were expressed in ng ml⁻¹ in terms of the NIH standard rat PRL-RP-1, whose potency is 11.0 IU mg⁻¹. The sensitivity of the PRL assay is 1.0 ng ml⁻¹; intra-assay variation was 5% while repeated assay of a reference plasma showed an intra-assay variation of 6%. To avoid possible intra-assay variations, all samples were assayed in a single RIA. The hypothalamus was dissected and GABA was assayed as described in Table 1 legend. Values represent the mean ± s.e.m. of at least five determinations.

**P* < 0.01 versus saline-injected rats by Student's *t*-test.

Detection of GABA at AP level extends the list of neurotransmitters which have been identified in this glandular tissue¹⁶. In the absence of a synthetic pathway in the AP, the function of GABA-T at the AP level would be mainly to catabolise GABA released from CNS centres and then transported through the hypophyseal portal circulation. The high GABA-T activity found in the AP could readily serve this function. However, in view of the maintenance of GABA titres, although low, in the ectopic gland, we cannot exclude the possibility that a fraction of the GABA measured in the AP gland *in situ* may derive from the systemic circulation.

Table 4 Effect of i.v.t. administration of EOS on the PRL rise induced by blockade of pituitary DA receptors with domperidone

Treatment	Plasma prolactin (ng ml ⁻¹)		
	Δt ₃₀	Δt ₆₀	Δt ₉₀
Saline + DOM (8)	23.0 ± 5.6	25.8 ± 6.2	26.8 ± 5.7
EOS + DOM (7)	13.1 ± 3.4	13.6 ± 5.0	10.0 ± 2.3*

Sprague-Dawley male rats (200–220 g) were implanted via the lateral ventricle of the brain³² and prepared with chronic indwelling jugular cannulae inserted into the right atrium³⁹. After 2–3 days for recovery, they were given EOS (2 mg per kg in 10 µl saline) or saline injection and 3 h later received an i.v. injection of domperidone (DOM, Motilium, Janssen) 0.01 mg per kg, and were sampled from the jugular cannula for RIA determination of plasma PRL. Values refer to incremental changes (Δ) in plasma PRL (mean ± s.e.m.) recorded at various times after DOM administration (30, 60 and 90 min). Numbers of rats are indicated in parentheses. Baseline PRL levels were 7.8 ± 1.5 (mean ± s.e.m.) and 3.8 ± 0.7 ng ml⁻¹ (*P* < 0.05) in saline + DOM and EOS + DOM-injected rats, respectively. Calculation of the areas subtended by PRL profiles revealed a significant difference between saline + DOM and EOS + DOM treated groups (181 ± 19 mean ± s.e.m. versus 122 ± 16, respectively, *P* < 0.05 by Student's *t*-test).

**P* < 0.05 versus saline + DOM injected rats by Student's *t*-test.

The organisation of GABAergic pathways in the hypothalamus provides neuroanatomical evidence for an action of GABA exerted directly at the AP level. In addition to hypothalamic GABAergic fibres projecting to the MBH, there is

evidence for the existence of a medial basal hypothalamic (tubero-infundibular) pathway which projects to the ME²⁵. Experiments reported here involving i.v.t.-injected EOS suggest that the increase in GABA content at the AP level was the consequence of inhibition of GABA-T in this pathway with ensuing GABA loading in the stalk circulation. The time-related rise in AP GABA concentration after EOS strongly suggests this mechanism. GABA once released into the portal circulation would be competent to control in an inhibitory fashion PRL secretion from the AP, acting at the level of specific GABA receptor sites¹⁰. Since EOS, in contrast to muscimol⁶, is unable to affect GABA receptor sites directly¹⁷, the above is the more plausible explanation for the PRL-lowering effect of the centrally administered compound. Our data suggest that the inhibitory effect of GABA on plasma PRL occurs independently of DA changes.

The finding of GABA in the posterior lobe of the pituitary, and the increases in GABA titres after blockade of GABA-T confirm previous data²⁶ and may reflect a functional role for the amino acid at this level. A functional role for DA present in the neuro-intermediate lobe has been recently postulated²⁷.

The findings reported here underline the functional analogy between the anatomical and biochemical organisation of GABA and DA at ME-AP level, and suggest an important role for the amino acid in the control of PRL secretion.

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Dopamine receptors in the retina may all be linked to adenylate cyclase

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Dopamine seems to be the principal catecholamine in the vertebrate retina, where it is found in two types of neurone¹. In most species, there is a subclass of amacrine cell which makes synapses on other amacrine cells in the inner plexiform layer². In teleost fish and New World monkeys, the dopaminergic neurones are interplexiform cells which make synapses in both plexiform layers on horizontal, bipolar and amacrine cells³. In other dopamine-rich areas of the central nervous system, there is evidence for multiple dopamine receptor types^{4–7}, and two sub-types have been suggested⁸. D-1 receptors are associated with an adenylate cyclase system whereas D-2 receptors are not. D-2 receptors are characterised by a high affinity for the butyrophenones haloperidol⁹ and spiperone¹⁰, but these ligands also apparently bind to D-1 receptors¹¹. The butyrophenone domperidone also binds to central dopamine receptors¹², but it is a very weak antagonist of dopamine-sensitive adenylate cyclase¹³ (Table 1), suggesting that it may bind only to D-2 receptors. In the retina of many species, a dopamine-sensitive adenylate cyclase has been demonstrated^{14,15}, indicating the presence of D-1 receptors. We report here that there is no significant receptor-specific binding of ³H-domperidone to retinal membranes, thus suggesting the absence of D-2 receptors in this tissue.

The dopamine-sensitive adenylate cyclase in the retina seems to be identical to that found elsewhere in the brain. Figure 1 shows, for example, adenylate cyclase activity in homogenates of the retina and corpus striatum of the guinea pig prepared in similar conditions. In both homogenates, dopamine elicits a dose-dependent stimulation of cyclic AMP formation, with maximal stimulation occurring at concentrations of 0.1–1 mM, and half-maximal stimulation with approximately 10 μM dopamine. When expressed in terms of cyclic AMP produced above baseline levels, an average maximal response of 38.3 ± 6.7 pmol cyclic AMP per mg protein per min was obtained in four striatal homogenates, whereas a value of 47.3 ± 4.6 pmol per mg protein per min was obtained in seven retinal homogenates. These results indicate that there are substantial

Table 1 Inhibition of dopamine-stimulated adenylate cyclase in homogenates of guinea pig and carp retinae and rat striatum

Drug	K _i values (M)		
	Guinea pig retina	Carp retina	Rat striatum
Fluphenazine	3.9 × 10 ⁻⁹	3.0 × 10 ⁻⁹	4.3 × 10 ⁻⁹
α-Flupenthixol	4.8 × 10 ⁻⁹	5.1 × 10 ⁻⁹	1.0 × 10 ⁻⁹
(+)Butaclamol	2.4 × 10 ⁻⁸	2.5 × 10 ⁻⁸	8.8 × 10 ⁻⁹
Chlorpromazine	4.5 × 10 ⁻⁸	7.3 × 10 ⁻⁸	4.8 × 10 ⁻⁸
Haloperidol	9.0 × 10 ⁻⁸	9.4 × 10 ⁻⁸	9.1 × 10 ⁻⁸
(-)Butaclamol	>1 × 10 ⁻⁶	>1 × 10 ⁻⁶	>1 × 10 ⁻⁶
Domperidone	>1 × 10 ⁻⁶	>1 × 10 ⁻⁶	>1 × 10 ⁻⁶

The effect of each drug on dopamine-stimulated adenylate cyclase activity in homogenates of carp and guinea pig retina was examined at six concentrations in the range 0.01–100 μM. Data are the results from two or three separate experiments involving quadruplicate determinations and s.e.m. values of <10% of the mean. Drugs were added immediately before dopamine. K_i Values for each drug were calculated from the equation IC₅₀ = K_i(1 + S/K_m), where IC₅₀ is the concentration of drug required to produce 50% inhibition of the maximum dopamine-induced response, S is the concentration of dopamine used (100 μM) and K_m is the concentration of dopamine producing half-maximal stimulation of adenylate cyclase (guinea pig, 10 μM; carp, 1 μM); >1 × 10⁻⁶ M = less than 50% inhibition at 100 μM. Competitive inhibition has been assumed for all compounds tested. K_i values for rat striatum are taken from previous reports (refs 5, 13, 16).

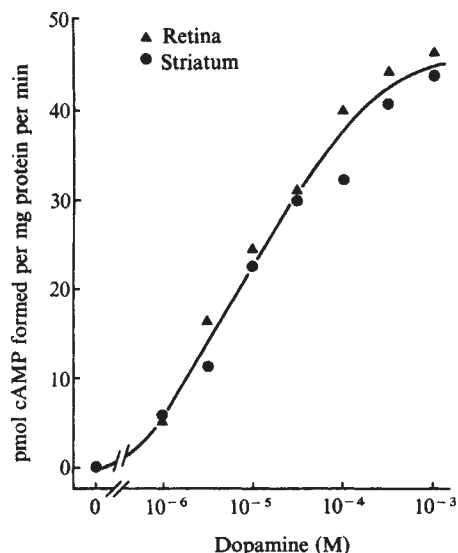


Fig. 1 Effect of dopamine on cyclic AMP production in homogenates of guinea pig retina and striatum. Results are expressed as pmol cyclic AMP per mg protein per min produced above baseline levels. Each point is the mean of three separate experiments involving quadruplicate determinations. s.e.m. values were less than 20% of the mean. Adenylate cyclase activity was assayed using the method of Kebejian *et al.*¹⁷ as follows. Retinae and striata were removed from guinea pigs (250–300 g) and separately homogenised in 2 mM Tris-maleate buffer containing 2 mM EGTA. Retinae were homogenised using a ratio of 1 retina to 500 μ l buffer, and striata using a ratio of 1 mg wet weight striata to 25 μ l buffer. Aliquots (20 μ l) of this homogenate were added to assay tubes containing 130 μ l of 80 mM Tris-maleate buffer (pH 7.4), 2 mM MgSO₄, 10 mM theophylline and 0.2 mM EGTA plus dopamine as indicated. The reaction in each tube was initiated by the addition of 50 μ l ATP to a final concentration of 1 mM. The tubes were incubated with shaking at 30°C for 3 or 5 min and transferred to a boiling water bath for 3 min followed by centrifugation. Duplicate 10 μ l samples of the final supernatant were assayed for cyclic AMP content by the method of Brown *et al.*¹⁸. Baseline cyclic AMP levels in four striatal homogenates were 70.6 ± 12.8 pmol cyclic AMP per mg protein per min and 19.4 ± 2.9 pmol cyclic AMP per mg protein per min in seven retinal homogenates. Protein contents were determined according to the method of Lowry *et al.*¹⁹ using bovine serum albumin as a standard.

amounts of dopamine-sensitive adenylate cyclase, and presumably D-1 receptors, in both guinea pig retina and striatum.

Furthermore, in guinea pig retinal homogenates, neither the α -adrenoreceptor agonist phenylephrine nor the β -adrenoreceptor agonist isoprenaline produce any significant stimulation of adenylate cyclase at concentrations up to 100 μ M, indicating that the catecholamine receptors linked to adenylate cyclase in the retina are specific for dopamine. In addition, various dopaminergic antagonists, including fluphenazine, α -flupenthixol, chlorpromazine and haloperidol, inhibit the retinal dopamine-sensitive adenylate cyclase with K_i values similar to those previously obtained in striatal homogenates⁵ (Table 1). The dopamine-sensitive adenylate cyclase in retinal homogenates is also antagonised by the neuroleptic agent (+)-butaclamol, whereas (–)-butaclamol is essentially inactive. This stereospecificity is also similar to that observed in striatal homogenates⁵. Finally, in agreement with results obtained in homogenates of rat striatum¹³, domperidone shows no significant antagonism of the retinal dopamine-sensitive adenylate cyclase at concentrations up to 100 μ M.

Significant binding of ³H-domperidone can be demonstrated readily in guinea pig striatal homogenates (Fig. 2), and 85–90% of this binding is displaced by non-radioactive dopamine at a concentration of 1 mM, with 50% displacement of specific binding at 1 μ M dopamine. These data indicate that there are many dopamine-specific binding sites for domperidone in the guinea pig striatum. In contrast, comparatively little ³H-

domperidone binding is detectable in similarly prepared homogenates of guinea pig retinae, and dopamine at concentrations up to 10 μ M fails to displace any of the bound drug. A small amount of displacement seems to occur with dopamine concentrations of 100 μ M and 1 μ M, but this effect is not statistically significant.

The amount of non-displaceable ³H-domperidone binding observed in retinal homogenates is comparable to the level of nonspecific binding remaining in striatal homogenates incubated with 1 mM dopamine, and probably represents binding of the ligand to non-receptor sites. In one homogenate of the rabbit retina there was a comparable small amount of ³H-domperidone binding (2.5 fmol per mg wet weight tissue) which was also not affected by dopamine at concentrations up to 1 mM.

We have also studied the dopamine-sensitive adenylate cyclase and ³H-domperidone binding in the carp retina, whose dopaminergic neurones are of the interplexiform type³. As in the guinea pig, a prominent dopamine-sensitive adenylate cyclase is present in this retina. This cyclase also seems to be identical to those in other retinae and other brain regions in terms of its specificity for dopamine, sensitivity to various neuroleptic drugs but insensitivity to domperidone (Table 1). As in the other retinae examined, no dopamine-displaceable ³H-domperidone binding was observed in homogenates of the carp retina in five experiments. However, in carp, the amount of non-displaceable binding was approximately twice that found in the guinea pig (4.4 fmol, per mg wet weight compared with 2.8 fmol per mg wet weight).

In preliminary experiments, we have examined the binding of another butyrophenone derivative, ³H-spiperone, to guinea pig and carp retinal homogenates. This ligand apparently binds to both D-1 and D-2 receptors in striatal homogenates¹¹. In

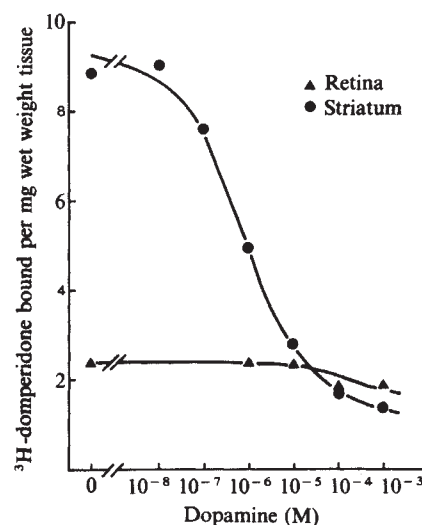


Fig. 2 Effect of dopamine on the binding of ³H-domperidone to guinea pig striatal and retinal homogenates. Binding of ³H-domperidone (corrected for filter blanks) to guinea pig striatal or retinal membranes, in the absence or presence of various concentrations of dopamine, is expressed as fmol bound per mg wet weight tissue. Each point represents the mean of four experiments involving triplicate determinations. s.e.m. values were less than 15% and 30% of the mean in the striatum and retina, respectively. Membrane preparations were prepared as previously described^{10,20,21}. Incubation tubes containing 50 μ l ³H-domperidone (Janssen, specific activity 10 Ci mmol⁻¹) to give a final concentration of 1 nM, 50 μ l dopamine (final concentration as indicated) or distilled water and 900 μ l homogenate (equivalent to ~5 mg wet weight of original tissue for striatum and 7–10 mg wet weight of original tissue for retina) were incubated for 20 min at 30°C. Membranes were then collected by filtration, washed, and the bound radioactivity determined^{10,20,21}. Total binding of ³H-domperidone was 8.87 ± 0.16 and 2.38 ± 0.38 fmol per mg wet weight tissue in striatum and retina, respectively. In the presence of 1 mM dopamine, this binding was reduced to 1.38 ± 0.11 and 1.86 ± 0.39 fmol per mg wet weight tissue.

agreement with this, we have observed a significant amount of dopamine-displaceable ^3H -spiperone binding in both these retinæ.

In conclusion, our results suggest that the retina may represent an area of the vertebrate central nervous system which contains a pure population of D-1 receptors; that is, all linked to adenylate cyclase. This situation apparently holds regardless of the type of dopaminergic neurone present in the retina. Thus, whether the dopaminergic neurones are a class of amacrine cell, as in the guinea pig or rabbit, or interplexiform cells, as in the carp, no D-2 dopamine receptors seem to be present. These results further imply that all neurones postsynaptic to the retinal dopaminergic cells, namely amacrine cells in the guinea pig and rabbit, and bipolar, horizontal and amacrine cells in the carp, possess a dopamine-sensitive adenylate cyclase.

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Combined effects of adrenaline and insulin on active electrogenic $\text{Na}^+ - \text{K}^+$ transport in rat soleus muscle

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Both β_2 -adrenoreceptor stimulants (such as adrenaline and salbutamol) and insulin can increase active $\text{Na}^+ - \text{K}^+$ transport^{1–6} and hyperpolarise skeletal muscle cells^{1,2,7–9}. Thus, adrenaline and insulin, which are otherwise antagonistic regulators of several metabolic processes, have one action in common, namely, stimulation of active ion translocation. This is especially interesting as cyclic AMP stimulates $\text{Na}^+ - \text{K}^+$ transport², whereas a lowering of the cytoplasmic concentration of cyclic AMP has been proposed as an early signal in the action of insulin^{10,11}. Here we report the results of experiments in which the active $\text{Na}^+ - \text{K}^+$ transport and membrane potential (E_M) of rat soleus muscles were studied during the action of supramaximal doses of insulin and β_2 -adrenoreceptor stimulants, alone and in combination. We conclude that the stimulant action of insulin on active electrogenic $\text{Na}^+ - \text{K}^+$ transport is unlikely to be evoked by a lowering of the intracellular concentration of cyclic AMP.

All experiments were carried out using the rat soleus muscle. The techniques for measuring $\text{Na}^+ - \text{K}^+$ transport and E_M have

been described in detail previously^{2,6,12}. For the measurements of the content and transport of Na^+ and K^+ , muscles obtained from rats weighing 60–70 g were used. It was earlier demonstrated that there is no significant difference between the E_M of muscles from small (60–70 g) and large (200–400 g) rats². As large fibres allow more stable recordings, the major part of the present measurements *in vitro* and *in vivo* were carried out using animals weighing 200–400 g.

In vivo recordings of E_M were made using pentobarbitone-anaesthetised rats weighing 300–400 g. In these experiments, the dorsal surface of the right soleus muscle was exposed at the bottom of a 'pool' formed by lifting the edges of the skin incision. The muscle surface was covered by a thin layer of standard Krebs–Ringer bicarbonate buffer on which a light paraffin oil was layered. The temperature of the pool and the body of the animal was maintained at 37 °C using a thermostated heating blanket. Venous blood samples were taken through a catheter in the left superior vena cava and drugs infused through a catheter in the left femoral vein. A Ag/AgCl reference electrode was buried in the left thigh muscles (the details of this technique are described elsewhere¹³).

Supramaximal concentrations of either adrenaline (10^{-5} M) or insulin (100 mU ml^{-1}) stimulated ^{22}Na efflux and ^{42}K influx (Table 1), and increased E_M within approximately the same interval of time (Table 2). These large doses were used to ensure that maximal stimulation was evoked by each agent alone. The effects of insulin were somewhat less pronounced and slower in onset than those of adrenaline. Ouabain (10^{-3} M) blocked the hyperpolarising effect of both hormones (Table 2), indicating that this is related to a stimulation of active $\text{Na}^+ - \text{K}^+$ transport. The effects of adrenaline and insulin were partially additive on $\text{Na}^+ - \text{K}^+$ transport, whether the insulin was added before, after or simultaneously with adrenaline (as in Table 1). On the other hand, although the addition of adrenaline to muscles already exposed to insulin gave a further hyperpolarisation, this was smaller than that produced by adrenaline alone. Moreover, the hyperpolarisation produced by adrenaline was significantly reduced by the later addition of insulin. Similar results were obtained with salbutamol alone or combined with insulin (Table 2).

Also *in vivo*, salbutamol and adrenaline produced a hyperpolarisation^{13,14}. This was dose dependent and accompanied by a small reduction of plasma K^+ . The hyperpolarisation produced by a supramaximal salbutamol concentration *in vivo* was larger than that evoked by a similar concentration *in vitro*. Insulin, whether released in response to the intravenous (i.v.) injection of glucose (Table 3) or injected i.v., also produced a hyperpolarisation and a non-significant fall of plasma $[\text{K}^+]$. The i.v. injection of 0.5 IU insulin to a 250-g rat produced a hyperpolarisation of 15.2 mV (from -69.9 ± 0.6 [39 observations] to -85.1 ± 0.5 [44]) which was seen in all four fibre layers investigated. The much larger response to exogenous insulin is poss-

Table 1 Effect of insulin and adrenaline on ^{22}Na washout and ^{42}K uptake in rat soleus muscle

	^{22}Na Efflux (min^{-1})	<i>P</i>	^{42}K Influx ($\mu\text{mol per g per min}$)	<i>P</i>
Control	0.058 ± 0.002 (5)		0.54 ± 0.02 (8)	
Insulin (100 mU ml^{-1})	0.081 ± 0.004 (6)	<0.001	0.65 ± 0.03 (8)	<0.005
Insulin (100 mU ml^{-1}) + adrenaline (10^{-5} M)	0.103 ± 0.004 (6)	<0.005	0.81 ± 0.02 (8)	<0.001
Adrenaline (10^{-5} M)	0.098 ± 0.004 (6)	>0.10	0.71 ± 0.02 (8)	<0.01

For experimental details, see refs 2 and 6. The rate of ^{22}Na washout is given as the fraction of ^{22}Na activity lost from the tissue per min, and ^{42}K uptake as the amount of ^{42}K activity gaining access to the tissue compartment not available to ^{14}C -sucrose. The measurements were carried out within the first 10 min of exposure to the hormones, and all results are given $\pm$ s.e.m. with the number of observations in parentheses.

Table 2 The action of insulin and adrenaline on the E_M of rat soleus muscle

Muscle (i)	Additions	ΔE_M (mV)	n	P
a	Insulin	$+3.5 \pm 0.2$	(199/5)	<0.001
	Insulin + adrenaline	$+7.3 \pm 0.3$	(192/5)	
b	Adrenaline	$+7.4 \pm 0.4$	(194/5)	<0.001
	Adrenaline + insulin	$+6.0 \pm 0.3$	(182/5)	
a	Insulin	$+3.6 \pm 0.4$	(151/4)	<0.001
	Insulin + salbutamol	$+9.3 \pm 0.4$	(146/4)	
b	Salbutamol	$+9.1 \pm 0.4$	(144/4)	<0.10
	Salbutamol + insulin	$+8.1 \pm 0.4$	(155/4)	
(ii)				
a	Insulin	$+4.3 \pm 0.4$	(171/4)	<0.001
b	Ouabain + insulin	-0.5 ± 0.4	(172/4)	
a	Adrenaline	$+5.8 \pm 0.7$	(115/5)	<0.001
b	Ouabain + adrenaline	-0.9 ± 1.0	(136/5)	

(i) The action of insulin (100 mU ml⁻¹) and adrenaline (10⁻⁵ M), or insulin (100 mU ml⁻¹) and salbutamol (10⁻⁵ M), applied individually or in combination, on the E_M of rat soleus muscle fibres (animal size 200–400 g). Paired experiments were carried out in which insulin was added either before (muscles *a*) or after (muscles *b*) the β_2 -adrenoreceptor agonist (using contralateral muscles of the same rats). A series of penetrations was first made while the muscle was bathed in drug-free Krebs–Ringer bicarbonate buffer (control). A further series of penetrations was started when the maximum drug-induced hyperpolarisation had been reached, that is, about 7 min after the bath concentration of β_2 -adrenoreceptor agonist was brought to 10⁻⁵ M (ref. 2). In the experiments with insulin, the maximum rise was only reached 15 min after the onset of exposure. Although the ion contents of the muscle as a whole continued to change after these time intervals, no further change of potential was noted. The mean ($\pm$ s.e.m.) changes in E_M produced by drug addition, compared with the drug-free control E_M , are tabulated (ΔE_M). *n* Indicates the number of fibres penetrated and the number of muscles investigated.

(ii) The action of insulin (100 mU ml⁻¹) and adrenaline (6 × 10⁻⁶ M) on the E_M of rat soleus muscle fibres in the absence and presence of ouabain (10⁻³ M). Paired experiments were carried out in which insulin was added to the soleus muscles of 250-g rats individually, or after the muscles had been pretreated with ouabain (contralateral muscles). This lasted 30 min to allow time for ouabain-induced muscle fibrillations to cease. In a corresponding series carried out on 60-g rats adrenaline was added alone or after ouabain pretreatment. The penetrations were timed as in (i). The *P* values given express the significance of difference between ΔE_M values obtained with one agent alone or two agents combined. Except in the presence of ouabain, all test values were significantly different (*P* < 0.001) from the control values obtained in drug-free solution.

Table 3 Effect of i.v. administration of glucose on serum K⁺, serum insulin and E_M of rat soleus muscle *in vivo*

	Control $\pm$ s.e.m.	10–20 min after glucose i.v. $\pm$ s.e.m.	Δ	<i>n</i>	<i>P</i>
Serum [K ⁺] (mM)	4.2 ± 0.2	3.9 ± 0.1	-0.3	8/4	>0.10
Serum insulin (μ M ml ⁻¹)	20 ± 3	179 ± 16	+159	4/2	<0.001
E_M (mV)	-70.3 ± 0.6	-75.8 ± 0.6	+5.5	130/4	<0.001

Female fed rats weighing 300–400 g were anaesthetised with pentobarbitone and the dorsal surface of the right soleus muscle exposed to allow the recording of E_M . Following a control period, each animal was given an i.v. injection of D-glucose (1 mg per g body weight) and the changes in serum K⁺, serum immunoreactive insulin and E_M were recorded. Control experiments in which mannitol (1 mg per g body weight) was given instead of glucose showed no significant change in any of these parameters. *n* Indicates the number of observations and the number of animals.

ibly related to the hypoglycaemia and the ensuing catecholamine release from the adrenals. *In vivo*, the combined action of adrenoreceptor agonists and insulin was additive. In two animals (250 g) the injection of 50 μ g salbutamol produced a hyperpolarisation of 12.2 mV (from -66.2 ± 0.6 [40 obser-

Table 4 Effect of adrenaline and insulin on Na⁺–K⁺ contents in isolated rat soleus muscle

	Na ⁺ Content (μ mol per g wet weight)	K ⁺ Content (μ mol per g wet weight)
Control	10.9 ± 0.4 (7)	77.5 ± 1.2 (7)
Adrenaline (10 ⁻⁵ M)	5.9 ± 0.5 (6)	84.1 ± 0.8 (6)
Adrenaline (10 ⁻⁵ M) + insulin (100 mU ml ⁻¹)	3.7 ± 0.4 (6)	94.1 ± 1.6 (6)
Insulin (100 mU ml ⁻¹)	8.5 ± 0.5 (7)	85.1 ± 1.7 (7)

Groups of six or seven muscles were incubated for 90 min in 3 ml of Krebs–Ringer bicarbonate buffer containing 1 mM ¹⁴C-sucrose with or without the additions indicated. They were then blotted, homogenised in 4 ml 5% trichloroacetic acid and the extract centrifuged. Aliquots of the clear supernatant were taken for flame photometry and measurement of ¹⁴C activity. The Na⁺–K⁺ contents of the tissue compartments not available to ¹⁴C-sucrose were calculated and expressed as μ mol per g tissue wet weight. The results are given as mean $\pm$ s.e.m. with the number of observations in parentheses.

vations/2 muscles] to -78.4 ± 0.5 [52/2]). The subsequent injection of 1 IU of insulin produced a further 4.6 mV hyperpolarisation (to -83.0 ± 0.5 [64/2]).

As demonstrated earlier, stimulation of the active Na⁺–K⁺ transport, whether induced by insulin⁶ or adrenaline², leads to a drop in the intracellular Na⁺–K⁺ concentration ratio (Table 4). Again, the actions of the two hormones on Na⁺–K⁺ distribution seems to be additive, indicating that even during a long-term exposure, their respective sets of cellular signals do not interfere with each other.

There is strong evidence that among the cellular signals initiated by adrenaline, cyclic AMP is important in eliciting a variety of effects produced by β_2 -adrenoreceptor stimulation. Both cyclic AMP and dibutyryl cyclic AMP (in combination with the phosphodiesterase inhibitor, theophylline) stimulate the active electrogenic Na⁺–K⁺ transport². Our observations are not readily compatible with the assumption that the action of insulin on Na⁺–K⁺ transport (judged by the measurement of active ion fluxes and cell Na⁺–K⁺ content) is secondary to a decrease in the concentration of cytoplasmic cyclic AMP. It seems reasonable to conclude that the system mediating active Na⁺–K⁺ transport is sensitive to two hormonal regulators sharing a final common path (see ref. 15).

The finding that a combination of β_2 -adrenoreceptor agonist plus insulin can produce a hyperpolarisation that is significantly smaller than that of β_2 -adrenoreceptor agonist alone does not correlate with the studies of ion transport and contents. This discrepancy may be explained if insulin not only induces a stimulation of a 3:2 coupled active Na⁺–K⁺ transport⁶ but also increases the membrane permeability to Na⁺ (ref. 8). A permeability increase could thus 'shunt' electrogenic ion translocation, or directly depolarise the membrane. However, we cannot explain why a change in the sequence of addition of insulin and adrenaline can produce significantly different results.

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Contractions of cat small intestinal smooth muscle in calcium-free solution

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A rise in internal Ca^{2+} is the signal which activates the contractile mechanism in muscle. In striated muscle there is a well developed sarcoplasmic reticulum which stores and releases the bulk of the Ca^{2+} involved in the contraction cycle^{1,2}, allowing the muscle to operate relatively independently of external calcium levels. In smooth muscle the sarcoplasmic reticulum system is much less extensive^{3,4} and normal contractile activity is very sensitive to calcium levels in the bathing medium⁵⁻⁷. These findings, together with the small size of smooth muscle cells which minimises diffusion time from the plasma membrane to the contractile apparatus, have suggested to some investigators that the Ca^{2+} which enters the cell during Ca^{2+} -mediated action potentials is either sufficient by itself to activate contraction or is a necessary trigger for the further release of Ca^{2+} from internal stores⁸⁻¹¹. This notion is given support by the observation that contractile activity rapidly dies out when smooth muscle preparations are exposed to calcium-free solutions. However, electrical activity is eventually lost in these solutions. We report here on spontaneous contractile and electrical activity, and evoked mechanical activity, recorded after prolonged exposure to calcium-free EGTA saline in intestinal smooth muscle of cat.

Segments of small intestine were removed from cats anaesthetised with α -chloralose and placed immediately into Krebs solution of the following composition (mM): NaCl, 118.5; KCl, 4.7; MgCl_2 , 1.2; NaHCO_3 , 23.8; KH_2PO_4 , 1.2; CaCl_2 , 2.5, and glucose 5.5 (ref. 12). External electrical recording techniques were used¹¹. Mechanical recordings were made with an isometric tension transducer (Grass Instruments model FTO3C). Experiments were performed at 35–37 °C.

Normal electrical activity in cat small intestine consists of slow rhythmic depolarisations (slow waves) with spike potentials often superimposed near the slow wave peak. The slow depolarisation is maintained for 2 to 4 s and peak amplitude recorded intracellularly ranges between 16 and 40 mV in normal musculature¹². Spike potentials seldom reach 0 mV in intracellular recordings. When gross contractions occur they are associated with spike activity.

Prosser *et al.*¹³ showed that normal electrical activity in cat small intestine was eliminated by incubation in Krebs solution containing no added calcium and 5–10 mM EGTA ('calcium-free Krebs'). Following periods of electrical quiescence which ranged from 10 to 40 min, the muscle developed prolonged potentials in the calcium-free Krebs solution. Intracellularly recorded, the potentials were approximately 40 mV in amplitude. Thus the prolonged potentials apparently result from sodium ions traversing channels normally used by calcium.

Mechanical and electrical events in control Krebs, during the period immediately after exposure to calcium-free Krebs and after incubation for 40 min in calcium-free Krebs, are shown in Fig. 1a, b, c. The prolonged potentials developed in approximately 40% of the preparations tested, and rhythmic contractions, such as those shown in Fig. 1c and 1d, were displayed by 90% of these spontaneous preparations. The mean amplitude of the EGTA contractions was 65% of the control contractions, although some were larger than control activity. Calcium-free Krebs flowed continuously through the chamber during experiments.

To exclude the possibility that calcium might be leached from the mucosa into the interstitial fluid and act as an extracellular calcium store to the muscle cell, 30 preparations in which the

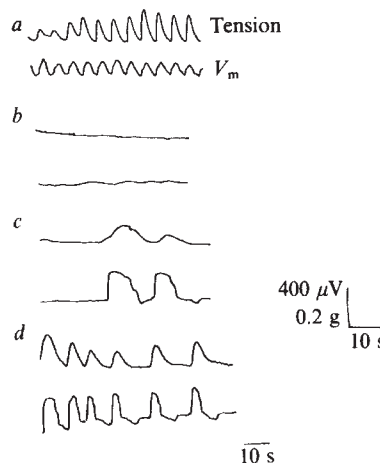


Fig. 1 a, Pressure electrode¹¹ recording of electrical activity (V_m , lower trace) and corresponding mechanical activity (upper trace) in control saline solution. b, Resulting activity following incubation for 7 min in calcium-free saline. c, Exposure for 40 min to calcium-free saline. d, Approximately 47 min exposure to calcium-free solution. Note change in time calibration.

mucosa was surgically removed were tested. The results of incubation of mucosa-free preparations in calcium-free saline were qualitatively the same as those obtained for intact preparations except the success rate of obtaining EGTA-induced activity was diminished in preparations devoid of the mucosa, possibly due to the more extensive manipulation of the tissue.

Electrical stimulation could induce contractions in preparations remaining quiescent in calcium-free solution (Fig. 2). In some cases these preparations developed spontaneous electrical and contractile activity after stimulation. In other quiescent preparations barium chloride was applied and induced spikes with accompanying contractions (Fig. 3). Either treatment was effective even after preparations had been in calcium-free saline for over 2 h.

In the barium experiments the possibility exists of direct activation of the contractile apparatus by barium. However, this seems unlikely in view of the relative concentrations involved. Ebashi *et al.*¹⁴ demonstrated that myosin B preparations from chicken gizzard and bovine aortic muscles are approximately 200 times less sensitive to barium than to calcium. Barium-induced contractions in calcium-free saline can be as large as control contractions, suggesting that internal barium levels would have to approximate 200 times internal calcium levels during peak contractions ($\sim 2 \mu\text{M}$ level for calcium-activated contractions). We would suggest, instead, that barium acts by supporting large action potentials¹⁵ and that the plasma membrane depolarisation results in a release of internal calcium. The action of barium would then be similar to direct electrical stimulation on preparations in calcium-free saline.

Barrett and Barrett¹⁶ have demonstrated the existence of a 'protected' extracellular compartment of calcium in frog skeletal muscle. The contents of this compartment were not removed by incubation of muscles in solutions with no added calcium plus 1 mM EGTA but were removed by high EGTA saline. To eliminate the possible presence of a 'protected' extracellular calcium compartment in cat small intestine, we incubated

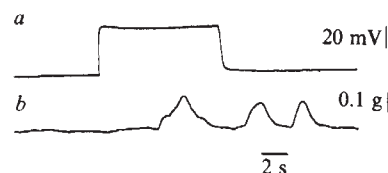


Fig. 2 Application of electrical stimulation to preparation remaining quiescent after exposure for 30 min to calcium-free saline. Preparation is mucosa free.

mucosa-free preparations in 10 mM EGTA for 20 min and then in 30–50 mM EGTA for 30 min. Contractions could be elicited by electrical stimulation or barium chloride for the first 10 min in high EGTA. The final muscle contraction in high EGTA did not relax and tension thereafter showed a slow, steady increase, indicating that the muscle may have gone into contracture. This state was observed for periods of up to 20 min but may have lasted significantly longer. High EGTA concentrations might have induced contracture by large membrane depolarisations¹⁶.

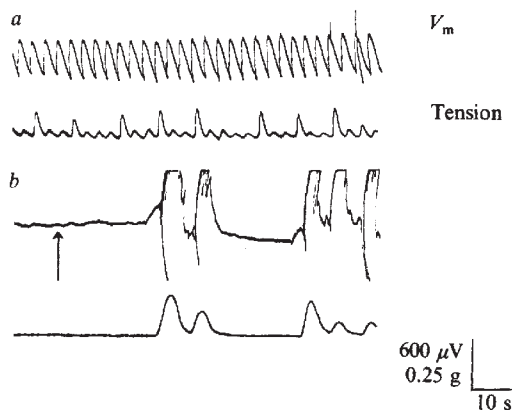


Fig. 3 *a*, Volume recording of electrical activity (upper trace) and corresponding mechanical activity (lower trace) in control saline solution from a mucosa-free preparation. *b*, Thirty seconds (at arrow) after the start of 3 mM BaCl₂ in calcium-free saline; the preparation was incubated in calcium-free saline for 175 min.

Our findings are similar to those of Somlyo *et al.*⁴ who observed spontaneous contractions of turtle oviduct that persisted for ~30 min in calcium-free saline (4 mM EGTA). These investigators concluded that intracellular as well as extracellular sources of calcium can contribute to activation of contraction in smooth muscle. In contrast, others have reported that rhythmic mechanical activity is eliminated by EGTA^{17,18}. It is possible that these observations were made during a time corresponding to the transition period between normal activity and EGTA-induced activity in our experiments.

In the calcium-free solutions it is unlikely that there is an appreciable inward-directed calcium gradient. The ability of prolonged potentials, barium spikes or electrical stimulation to induce contractile activity then suggests that an influx of calcium is not a prerequisite for contractions to occur and that internal stores of calcium which may be released by membrane depolarisation can support the contractile process. It is not clear to what extent this internal store of calcium participates in contractions in physiological conditions. Since the contractions we observed in calcium-free saline lasted for up to 1 h, it seems probable that a substantial portion of the activating calcium is recycled and therefore not readily accessible to extrusion mechanisms such as Na–Ca exchange.

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Control of cytoplasmic actin gel–sol transformation by gelsolin, a calcium-dependent regulatory protein

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The peripheral cytoplasm of macrophages is involved in the control of locomotion, secretion and endocytosis, events common to many eukaryotic cells. During these activities, the cortical cytoplasm, which contains numerous actin filaments^{1,2}, appears to undergo reversible gel–sol transformations³: cycles of gelation and solation are demonstrable in suitably prepared macrophage extracts, and the gels contain tangled actin filaments⁴. These changes in consistency of cytoplasmic actin may regulate motile events in the macrophage periphery. Calcium in micromolar concentrations prevents gelation of crude macrophage cytoplasmic extracts⁴, providing a possible link to abundant indirect evidence implicating calcium in the regulation of locomotion, secretion and endocytosis⁵. Similar calcium-sensitive gelation phenomena occur in crude cell extracts from diverse cell types and may have a relevance for control of cell movements in general^{6–11}. Actin gelation results from the cross-linking of actin filaments (F-actin) by other proteins. In macrophages, a high molecular weight actin-binding protein (ABP) is the principal actin cross-linking protein¹². Cross-linking of actin by these purified actin-binding proteins, however, is insensitive to changes in the calcium concentration^{4,12}, so that another factor must mediate the expression of a calcium effect. We have now isolated such a calcium-dependent regulatory protein from macrophages and call it gelsolin.

Rabbit lung macrophage cytoplasmic extracts, prepared in buffered sucrose solution containing protease inhibitors¹² and 5 mM EGTA, solidified to form gels when warmed in the presence of 0.1 M KCl. Addition of calcium chloride to micromolar-free calcium concentration inhibited gelation and caused solation of existing gels. Chelation of Ca²⁺ with EGTA restored gelation to the calcium-treated extract, ruling out calcium-dependent denaturation of the gelation components. The fluid expressed from gelled extract by centrifugation at 12,000g for 15 min at 25 °C, designated S2, did not gel by itself. However, it inhibited the gelation of rabbit muscle F-actin by purified macrophage ABP or chicken gizzard filamin in the presence of Ca²⁺. Excess EGTA restored gelation. The experimental conditions used to demonstrate the Ca²⁺-dependent regulator activity in S2 were as described in Table 1 for the purified gelsolin protein, and the results were qualitatively similar. The inhibitory activity in S2 was sensitive to trypsin digestion (1 mg ml⁻¹ for 30 min at 25 °C) and boiling at 100 °C for 1 min.

The regulatory activity in S2 was purified by sequential ion-exchange chromatography and gel filtration (Fig. 1). The gelation inhibitory activity eluted from a DEAE-Sepharose column in one symmetrical peak at KCl concentrations between 0.10 and 0.15 M, and eluted in a single symmetrical peak from a Sephadex G-200 column. Based on the elution of globular proteins with known molecular weights from this column, the regulator protein had an apparent molecular weight (MW) of 160,000. The purification recovered 26–69% of the gelation inhibitory activity present in S2 and enriched the specific inhibitory activity 36- to 61-fold. Purification of the calcium-dependent inhibitory activity coincided with the enrichment of a

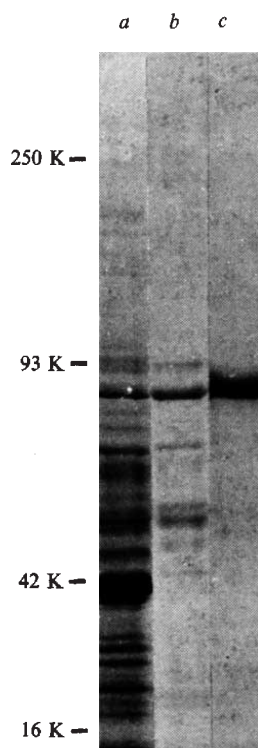


Fig. 1 Purification of gelsolin. All purification procedures were carried out in the cold. S2 (100 ml) was dialysed against 30 volumes of a solution containing 1 mM EGTA, 1 mM dithiothreitol, 0.5 mM ATP and 9 mM imidazole-HCl, pH 7.8. The dialysed sample was applied to a DEAE-Sepharose column (1.5 × 21 cm) equilibrated with the same buffer. The adherent proteins were eluted with a 500 ml linear 0–0.6 M KCl concentration gradient. Fractions with gelation inhibitory activity (determined as described below) were pooled and solid ammonium sulphate was added to a final concentration of 306 mg ml⁻¹. The precipitated proteins were collected by centrifugation at 15,000g for 10 min, dissolved in 1 ml of a solution containing 0.8 M KCl, 1 mM EGTA, 5 mM dithiothreitol, and 10 mM imidazole-HCl, pH 7.5, and dialysed against the same solution. This solution was used for equilibration and elution of a Sephadex G-200 column (1.6 × 90 cm) to which the sample was applied. Calcium-dependent inhibition of actin gelation by the eluted fractions was measured by the sedimentation assay of Brotschi *et al.*¹². Rabbit muscle F-actin (2 mg ml⁻¹ prepared as in ref. 14, and filamin (0.05 mg ml⁻¹, prepared as in ref. 13) were incubated with the fractions to be tested in a solution of 0.1 M KCl, 20 mM imidazole-HCl, pH 7.2, 0.045 mM CaCl₂, for 30 min at 25 °C. Samples were centrifuged at 10,000g for 10 min at 20 °C and protein concentrations of the supernatant were determined by the method of Lowry¹⁵. The difference in protein concentrations between the initial reaction mixture and the final supernatant was a measure of the amount of protein incorporated into the sedimented gel pellet. The concentration of cross-linker in the control sample (without inhibitor) was adjusted to sediment about 50% of the total actin by 10 min, and the sedimentation rate was constant with time over this period. A decrease in actin plus cross-linker sedimented in the presence of added test fractions indicated an inhibition of gelation. The specific inhibitor activity was computed as: (mg protein sedimented in control – mg protein sedimented in sample with test protein)/mg test protein. Proteins from each purification step containing inhibitory activity were boiled for 1 min in 8 M urea, 10% SDS, 2% 2-mercaptoethanol and resolved by electrophoresis in 5–15% polyacrylamide gradient slab gels in the presence of SDS¹⁶. *a*, Macrophage S2 extract, 300 µg; *b*, DEAE-Sepharose fraction, 300 µg; *c*, Sephadex G-200 fraction, 50 µg. The subunit MWs (×10⁻³) of standard proteins are indicated.

Table 1 Calcium-dependent reversible inhibition of actin gelation by gelsolin

Proteins	Treatment		Gelation %
	First incubation	Second incubation	
1. Actin, ABP	Ca ²⁺	—	100
2. Actin, ABP	EGTA	—	100
3. Actin, ABP, gelsolin	Ca ²⁺	—	0
4. Actin, ABP, gelsolin	EGTA	—	91
5. Actin, gelsolin	Ca ²⁺	—	0
6. Actin, gelsolin	EGTA	—	0
7. Actin, ABP, gelsolin	Ca ²⁺	EGTA	76
8. Actin, ABP, gelsolin	EGTA	Ca ²⁺	33
9. Actin, filamin	Ca ²⁺	—	100
10. Actin, filamin	EGTA	—	112
11. Actin, filamin, gelsolin	Ca ²⁺	—	0
12. Actin, filamin, gelsolin	EGTA	—	99
13. Actin, filamin, gelsolin	EGTA	Ca ²⁺	25

F-actin (2 mg ml⁻¹), actin cross-linking proteins (macrophage ABP, 0.024 mg ml⁻¹ prepared as in ref. 12, or chicken gizzard filamin, 0.045 mg ml⁻¹) and gelsolin (0.025 mg ml⁻¹) were incubated in a solution containing 0.1 M KCl, 20 mM imidazole-HCl, pH 7.2, either 0.07 mM CaCl₂ or 1 mM EGTA, for 30 min at 25 °C. Samples no. 8 and 13 contained 0.2 mM EGTA. In a second incubation (40 min), EGTA was added to sample 7 to a final concentration of 1 mM, and CaCl₂ was added to samples 8 and 13 to 0.5 mM. An equivalent volume of buffer was added to the remaining tubes. Actin cross-linking was quantitated by the sedimentation assay of Brotschi *et al.*¹². Data were pooled from three separate experiments, and gelation was expressed as per cent of that observed in paired control samples (without gelsolin). 100% gelation for the experiment shown in lines 1–6 was 0.7 mg ml⁻¹ after a 10 min centrifugation, 1.1 mg ml⁻¹ for line 8 and 0.9 mg ml⁻¹ for lines 9–13.

91,000-MW polypeptide resolved by SDS polyacrylamide gel electrophoresis. This polypeptide was the major Coomassie blue-stained protein of the pooled G-200 activity peak (Fig. 1c). We therefore concluded that the 91,000 polypeptides are subunits of gelsolin and, based on the gel filtration data, suggest that the native molecule contains two of these subunits. Purified gelsolin, like the inhibitory factor in S2, was heat-labile, and conferred reversible calcium sensitivity to the gelation of actin filaments by actin cross-linking proteins (Table 1). Gelation of rabbit muscle actin by purified rabbit macrophage ABP was insensitive to Ca²⁺ (lines 1 and 2). Actin gelation by macrophage ABP became sensitive to Ca²⁺ when gelsolin was included: gelation was inhibited completely in the presence of calcium (line 3) but not in the presence of excess EGTA (line 4). Gelsolin by itself did not cross-link actin (lines 5 and 6). The inhibitory effect of gelsolin was partially reversible; gelation was largely restored to a calcium-treated sample by subsequent addition of sufficient quantities of EGTA (line 8). Similar calcium-dependent inhibition of gelation occurred when filamin was used as the actin cross-linking agent (lines 9–13). Inhibition of gelation

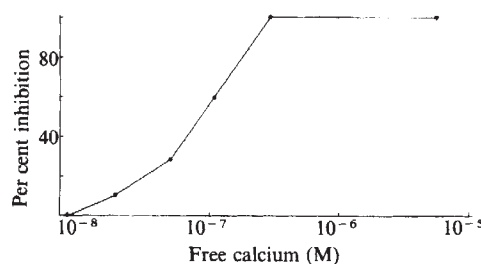


Fig. 2 Effect of calcium concentration on the inhibition of actin gelation by gelsolin. Gelsolin (0.03 mg ml⁻¹) was incubated with F-actin (2.0 mg ml⁻¹) and filamin (0.07 mg ml⁻¹) in a solution containing 0.1 M KCl, 20 mM imidazole-HCl, pH 7.2. The free Ca²⁺ concentration was varied by the use of Ca/EGTA buffers containing 0.4 mM EGTA, and calculated with the computer program of Perrin and Sayce¹⁷, as described by Potter and Gergely¹⁸.

Table 2 Effects of gelsolin on filamin-actin binding and actin sedimentability

Proteins	Conditions	Per cent of each protein sedimented			Weight ratios of sedimented proteins	
		Actin	Filamin	Gelsolin	Filamin:actin	Gelsolin:actin
1. Actin, filamin	Ca ²⁺	92	100	—	1:18	—
2. Actin, filamin, gelsolin	Ca ²⁺	82	100	47	1:16	1:55
3. Actin	Ca ²⁺	85	—	—	—	—
4. Actin, gelsolin	Ca ²⁺	71	—	47	—	1:48
5. Actin, filamin	EGTA	95	100	—	1:18	—
6. Actin, filamin, gelsolin	EGTA	93	100	18	1:18	1:163
7. Actin	EGTA	91	—	—	—	—
8. Actin, gelsolin	EGTA	91	—	15	—	1:192

F-actin (1.9 mg ml⁻¹) was incubated for 30 min at 25 °C with gelsolin (0.06 mg ml⁻¹) and filamin (0.1 mg ml⁻¹) in a solution of 0.1 M KCl, 20 mM imidazole-HCl, pH 7.2. Either 0.07 mM CaCl₂ or 0.4 mM EGTA was present. The tubes were centrifuged at 93,000g for 1.5 h at 4 °C. Equivalent aliquots of the initial mixtures and pelleted proteins were analysed by polyacrylamide gel electrophoresis in the presence of SDS¹⁶. The intensities of the Coomassie blue-stained bands were determined by scanning with a densitometer (E-Z Apparatus Corp), and the amounts of each protein sedimented were expressed as a percentage of that present in the initial mixtures.

occurred instantaneously, and the extent of inhibition was proportional to gelsolin concentrations. Assuming a MW of 90,000 for gelsolin subunit, the molar ratio of gelsolin subunit to actin monomers (MW 42,000) was 1:166, and gelsolin subunit to ABP or filamin (MW 500,000) was 1:0.45 when gelation was maximally inhibited.

Figure 2 shows the concentration of Ca²⁺ required for inhibition of actin gelation by gelsolin. These concentrations of free Ca²⁺ also inhibit gelation of cytoplasmic extract of macrophages and are within the limits observed in living cells.

To determine the site of action of gelsolin, we examined its effects on the binding of cross-linker proteins to actin, and on actin sedimentability and viscosity in the absence of cross-linking proteins. The binding of filamin and F-actin was determined from the extent of co-sedimentation of the two proteins at high centrifugal force. Filamin alone did not sediment. The amount of filamin which co-sedimented with F-actin was not changed after incubation with Ca²⁺ at a concentration of gelsolin in excess of that required to produce maximal inhibition of gelation (Table 2, lines 1, 2). In fact, because the sedimentability of actin decreased in the presence of gelsolin (compare lines 1 and 2), the filamin to actin weight ratios in the pellet increased slightly in the gelsolin-containing sample, compared with the control. Therefore, inhibition of gelation did not result from a decrease in the number of cross-linker molecules bound to the actin filaments.

A small decrease in actin sedimentability was also observed when actin was incubated with gelsolin and calcium in the absence of filamin (compare Table 2, lines 3 and 4). No change occurred in the presence of EGTA (compare lines 5 and 6, 7 and 8). A direct calcium-dependent effect of gelsolin on actin was demonstrable by measuring the viscosities of F-actin solutions. When an F-actin solution (2.0 mg ml⁻¹) was incubated with gelsolin (0.03 mg ml⁻¹), a concentration which completely inhibited gelation of actin by filamin, the specific viscosity, measured with Cannon-Manning microviscometers (size 100), decreased by 30% in the presence of Ca²⁺, but did not change in the presence of EGTA. The Ca²⁺-dependent drop in specific viscosity was equivalent to a decrease in F-actin concentration from 2.0 mg ml⁻¹ to 1.3 mg ml⁻¹.

Although the decreases in actin viscosity and sedimentability are consistent with shortening of actin filaments, it is clear from the small magnitude of the change that a substantial amount of gelsolin-treated actin remained in the polymerised state in conditions of complete inhibition of actin gelation.

Gelsolin may exert its effects by binding to actin in the presence of calcium. Gelsolin alone did not sediment under high-speed centrifugation. After incubation with F-actin and Ca²⁺, about half of the added gelsolin co-sedimented with actin (Table 2, line 4). Addition of filamin did not change the proportion of gelsolin co-sedimented with actin (line 2), suggesting that gelsolin bound directly to actin and not filamin. In these experiments, a large amount of gelsolin, in excess of that

required to dissolve actin gels, was used to render the polypeptide visible on stained polyacrylamide gels. Because of this excess, some of the added gelsolin remained in the supernatant. Furthermore, less actin sedimented in these conditions, and any gelsolin which might be bound to the non-sedimented actin would also remain in the supernatant. In the presence of EGTA, less than 20% of the gelsolin sedimented with actin, even though more actin was pelleted (Table 2, lines 6, 8). The ratios of pelleted gelsolin to actin were therefore much lower in the EGTA-treated samples than the calcium-treated samples.

These experiments have shown that gelsolin bound to actin in a Ca²⁺-dependent manner, and decreased the viscosity and sedimentability of actin in the absence of cross-linking proteins. Inhibition of actin gelation by gelsolin may therefore be mediated by a direct effect of gelsolin on actin filaments. To explain complete inhibition of actin gelation following limited decrease in actin viscosity and sedimentability, we propose that gelsolin divides actin filaments between cross-linking points, thereby increasing the number of filaments. This mechanism of disrupting cross-linked networks is consistent with the established theory for polymer gels, which predicts that small changes in the cross-linker to polymer ratio near a critical point can abruptly effect sol-gel transformations¹⁹. Actin ABP and actin-filamin networks behave in accordance with this theory^{12,20}. If gelsolin divides actin filaments, and the number of cross-links remains the same, the resulting increase in number of filaments effectively lowers the cross-link:polymer ratio, and causes dissolution of actin gels. The small number of gelsolin molecules required to inhibit gelation of actin is consistent with the idea that gelsolin can dissolve actin gels by causing a few breaks in actin filaments.

Our findings demonstrate that gelsolin, a heat-labile protein isolated from macrophages, dissolves actin networks in association with the limited breakdown of actin filaments. The expression of the regulatory function of gelsolin depends on variation in free calcium concentrations likely to occur in living cells. This is the first report of successful reconstitution of a Ca²⁺-controlled actin gelation system from purified proteins, similar to that described in cytoplasmic extracts from diverse sources. We conclude that gelsolin is a physiological calcium-dependent regulator of macrophage cytoplasmic structure.

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Neurectoderm markers retained in phenotypical skeletal muscle cells arising from a glial cell line

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Differentiation *in vitro* of striated muscle from apparently non-muscle precursor cells has been reported in thymus reticulum¹, a fibroblast-like mouse embryo line² and in a neurone-like cell line derived from a rat brain tumour³. Also Tomozawa and Sueko reported the differentiation of a peripheral neuro-tumour clonal stem cell line into separate neuronal and glial cell types⁴. We report here the reproducible and stable phenotypic change of a well characterised rat glial cell line, B₉ (refs 5-7), into multinucleate contractile skeletal muscle. The B₉ line was derived from a nitrosoethylurea-induced brain tumour, contains S-100 and 14-3-2 proteins, is electrically non-excitabile⁵ and has a putative glial-specific surface antigen, G2 (ref. 6). It has been phenotypically stable in its laboratory of origin at Salk Institute since 1973. After being transported to the Mayo Clinic in Minnesota the cells began to change from cuboidal to elongate shape, fuse into contractile multinucleate fibres and express nicotinic acetylcholine receptors (AChR) on their plasma membranes. Striated myofibrils appeared in their cytoplasm. The first sign of mesodermal differentiation in B₉ was the transitory appearance on its surface of Thy-1 which, in the rat, is a marker of thymocytes⁸, certain brain cell lines⁷, immature skeletal muscle^{9,10}, mammary myoepithelial lines¹¹ and fibroblasts¹². This antigen was not detected in previous studies at Salk Institute⁷. The inductive influence is not yet known. We propose that transformation of a neurectodermal line into muscle may be evidence of the capacity of mammalian neurectoderm to give rise to skeletal tissues. The concept that skeletal muscle in different anatomical regions might have different embryonic origins could be relevant to the distribution of muscles affected by certain diseases in man.

Glial lines B₉ and B₉₂ (ref. 5) and a myogenic line, L6, from rat limb muscle¹³, were transported by air at room temperature in plastic flasks containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), penicillin G and streptomycin. B₉ and L6 were collected by Viokase treatment (20%, 37 °C, 15 min), and passaged every 7 days at low density (1.5 × 10⁴ cells) in no. 3002 dishes (Falcon Labware) containing 5 ml DMEM with 10% FCS, penicillin G (20 U ml⁻¹) and streptomycin (0.02 mg ml⁻¹). B₉₂ was collected by vigorous

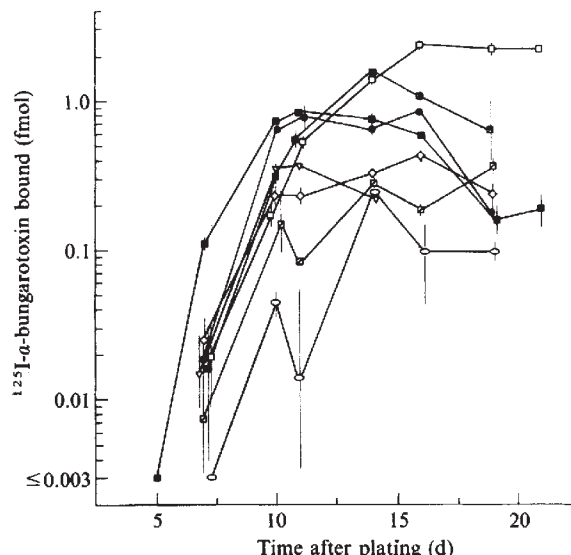


Fig. 1 Quantitation of nicotinic ACh receptors (AChR) on rat cell lines grown in microculture (2 × 10³ cells in 0.2 ml DMEM/FCS, Microtest II plates, Falcon). Specific binding of α-bungarotoxin (α-BT, prepared and labelled with 5 mCi ¹²⁵I per 100 μg as described elsewhere²²) was measured at multiple time points as follows. DMEM was withdrawn and 12 replicated wells washed once with HEPES-buffered DMEM (HBM) with 5% FCS. To 6 of the 12 wells was added 0.2 M carbamylcholine (CC). After 20 min at 21 °C an excess of ¹²⁵I-α-BT (4 × 10⁻⁹ M) was added to all 12 wells. Fluid was withdrawn after 90 min and the wells were washed three times with HBM/FCS, then one drop of 1 M KOH was added to each. After 15 min, the wells were swabbed with cotton-tipped applicators which were transferred to glass tubes for γ counting. Specific binding sites for ¹²⁵I-α-BT were calculated as: (mean c.p.m. without CC) - (mean c.p.m. with CC) × (mol standard ¹²⁵I-α-BT/c.p.m. standard ¹²⁵I-α-BT). Demonstration of antigenic determinants of nicotinic AChR in association with an α-BT binding macromolecule, extracted in non-ionic detergent from B₉M (subclone 33), distinguished this AChR from that of sympathetic ganglia type AChR¹⁴. Washed B₉M no. 33 cells (grown for 15 d in dishes of 150 cm² area) were extracted with 3% Triton X-100 (in 10 mM Tris, 100 mM NaCl, 1 mM EDTA, 5 × 10⁻⁴ M NaN₃ and 5 × 10⁻⁵ M PMSF). In the presence of an excess of ¹²⁵I-α-BT, a macromolecule binding α-BT was quantitated by chromatography on Sephadex G-100 (yield 2.34 fmol per cm² plate), as well as by precipitation from Triton X-100 extract²² by sequential addition of rat antibodies against *Torpedo* electric organ AChR and goat anti-rat Ig (yield 2.18 fmol per cm² plate). Cell lines shown include: ●, L6 (mesenchymal muscle); ■, B₉M (B₉-derived; muscle phenotype) and six subclones of B₉M: ▽, 13 (predominantly cuboidal; thin myotubes); ○, 15 (mixed, elongate and cuboidal); ◇, 20 (mixed, cuboidal and elongate; large myotubes); □, 27 (predominantly cuboidal; less fusion of elongates); ▤, 31 (mixed elongate and cuboidal; many multinucleate myotubes); □, 33 (predominantly elongate, large myotubes). Glial cell lines B₉₂ and B₉ are not shown, as they had no significant α-BT binding at any time (<0.01 fmol per well).

pipetting and similarly passaged. After the 7th passage, at 37 °C in humidified 12% CO₂/88% air, confluent cultures of B₉ were noted to have changed from cuboidal to fusiform shape. Many cells had fused to form multinucleate, vigorously contracting myotubes. At that time the laboratory had not received L6 or any other myogenic cell line. The changed cell line was renamed B₉M. Some cells were sent to California for continued passage and observation of phenotype; their progeny retained the skeletal muscle morphology on return to the original laboratory environment. Fresh stock of B₉ was sent from California for reference testing with B₉M and samples were cryopreserved after one subculture. After two passages in the new environment (with a different preparation of FCS) the second shipment of B₉ similarly became elongate and expressed pharmacological and antigenic markers of skeletal muscle (described below).

Cloning of B₉M at limiting dilution yielded 36 subclones (plating efficiency 47%). All were initially cuboidal; some became elongate and fused to form contractile myotubes; some remained predominantly cuboidal, but at high density all developed into a mixture of elongate and cuboidal. Specific binding sites for α-bungarotoxin (α-BT, indicative of AChR) were demonstrated on B₉M and on subclones of differing

Table 1 Free amino acid analyses of B₉, B₉M and L6

Amino Acid	B ₉	B ₉ M	L6
GABA	30; 23	27; 26	<0.1; <0.1
Ornithine	1.7; 2.1	2.4; 3.2	2.4; 3.2
Ethanolamine	4.0; 4.2	3.8; 4.2	<0.1; <0.1
Lysine	19; 21	19; 17	23; 22
Histidine	3.1; 4.3	3.7; 3.1	7.3; 8.9
Arginine	10; 9.0	9.0; 8.2	13; 15
Phosphoserine	3.1; 3.0	3.5; 3.8	7.6; 6.6
Phosphoethanolamine	60; 66	55; 60	46; 41
Taurine	116; 118	112; 117	92; 90
Aspartic acid	47; 49	48; 53	64; 61
Threonine	56; 61	66; 61	111; 105
Serine	20; 19	24; 29	34; 39
Asparagine	69; 64	65; 60	89; 86
Proline	36; 36	32; 33	64; 70
Glutamic acid	196; 186	201; 192	125; 123
Glycine	148; 155	145; 140	123; 135
Alanine	133; 131	130; 135	71; 65
Valine	8.3; 8.5	7.1; 8.6	27; 19
Isoleucine	6.6; 8.6	9.1; 8.0	18; 24
Leucine	9.6; 8.2	10; 11	31; 25
Tyrosine	5.2; 6.5	8.0; 7.2	20; 23
Phenylalanine	18; 15	16; 17	32; 38
β -Alanine	1.9; 2.3	2.1; 3.0	<0.1; <0.1

Analyses are expressed as residues per 1,000. Free amino acids were extracted as trichloroacetic acid-soluble residues from two separate preparations of each cell line in the exponential growth phase as described previously¹⁶. The content of GABA and β -alanine in B₉ and B₉M is characteristic of neuroectodermally derived cells; lack of these two amino acids in L6 is characteristic of cells of mesodermal origin¹⁶. Although significant differences are apparent in the content of several other amino acids in the B₉ and L6 lines, these differences are not found consistently between other cells of neuroectodermal and mesodermal origin.

morphology (Fig. 1). Subclones giving rise to abundant myotubes bound more α -BT. Myotubes of no. 33 were widest in diameter and in repeated experiments this subclone had approximately four times more AChR than the standard muscle

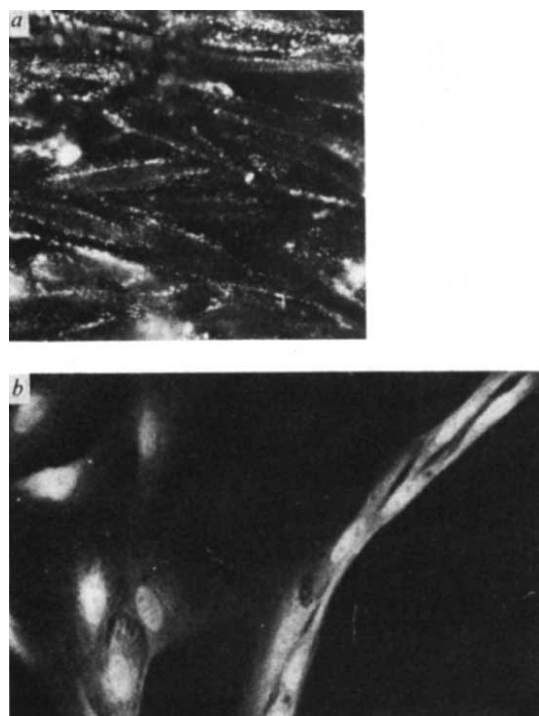


Fig. 2 For assay of Thy-1 by indirect immunofluorescence, cells were plated at 10^4 ml^{-1} into Petri dishes (1005, Falcon) containing round glass coverslips (no. 1 CGS, Propper Manufacturing Co.). ATS was used at a dilution of 1/50 followed by goat anti-rabbit IgG at 1/200 (Cappel Laboratories). *a*, In the living state the surfaces of elongate forms of subclone 33 of B₉M were Thy-1 positive. The characteristic coarse speckling of Thy-1 glycoprotein on developing myotubes in 10-day cultures is most prominent on cells of narrow diameter ($\times 1,512$). *b*, Acetone fixation revealed immunoreactive Thy-1 in the cytoplasm of cuboidal forms of the parental B₉M line and in aligned elongate pre-fusion cells 72 h after plating. Thy-1 glycoprotein is concentrated in the region of the nucleus in cuboidal forms and is more uniformly distributed in elongate forms ($\times 1,512$).

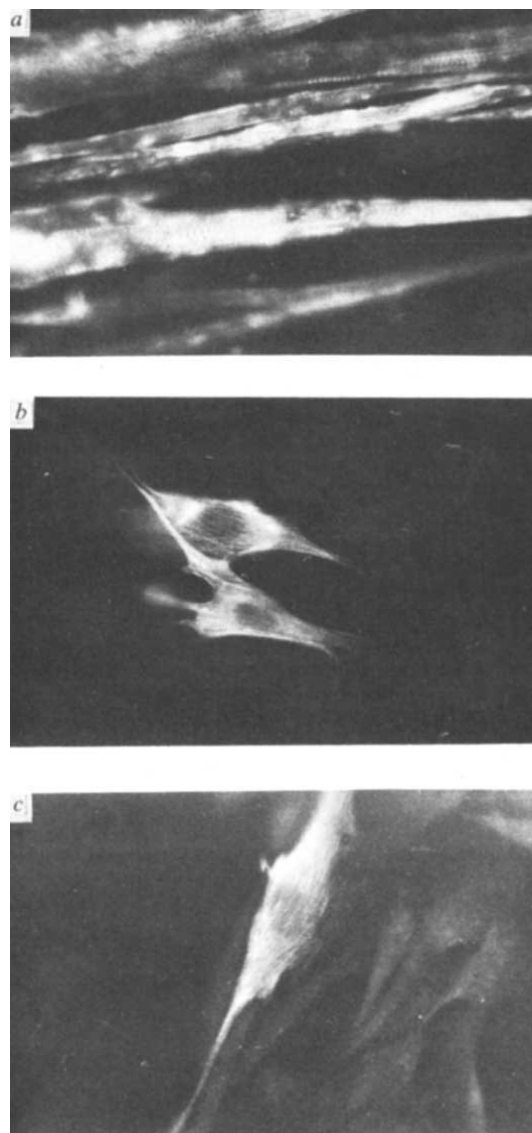


Fig. 3 Striated myofibrils characteristic of skeletal muscle were demonstrated in cytoplasm using serum (dilution 1/100) containing spontaneous autoantibodies reactive with contractile elements of skeletal muscle (obtained from a patient with myasthenia gravis). Indirect immunofluorescence used fluoresceinated goat anti-human IgG (Cappel Laboratories). *a*, Multinucleate L6 myotubes after 10 days in culture ($\times 1,512$); *b*, two aligned elongate B₉M cells after 72 h in culture ($\times 1,512$); *c*, positive elongate B₉M cell contrasts with negative background cells of more cuboidal morphology after 72 h in culture ($\times 1,512$).

line L6. Neither glial line B₉₂ nor the original B₉ bound ^{125}I - α -BT. Demonstration of antigenic determinants of skeletal muscle-type AChR in association with an α -BT-binding macromolecule extracted from B₉M (Fig. 1 legend) distinguished this receptor from that of sympathetic ganglia-type AChR¹⁴.

Thy-1 glycoprotein was detected by indirect immunofluorescence using rabbit anti-rat thymocyte serum (ATS) described elsewhere⁹. No cell line exhibited fluorescence with ATS which had been absorbed by rat brain. In earlier studies of L6 and primary rat muscle cultures⁹ we found Thy-1 on the surface of myoblasts and myotubes in early stages of fusion, but not on multinucleate myotubes. In the present study, L6 myoblasts, elongate forms of B₉M and its subclones and cuboidal cells contiguous with elongate forms were unequivocally positive (Fig. 2*a*). Acetone fixation revealed immunoreactive Thy-1 in the cytoplasm of cuboidal forms of B₉M (Fig. 2*b*). Small myotubes of B₉M and its subclones were most strongly positive for Thy-1; Thy-1 fluorescence on intermediate-sized myotubes was reduced (Fig. 2*a*) and multinucleate forms in 10-day cultures

lacked detectable Thy-1. Recently transported B₉ lacked Thy-1, but after several passages, a few elongate Thy-1-positive cells appeared on top of the Thy-1-negative cuboidal monolayer.

Sera from certain patients with myasthenia gravis contain antibodies which react with contractile elements of striated muscle¹⁵. A myasthenia gravis patient's serum with this reactivity revealed cytoplasmic filaments in some mononucleate B₉M and L6 cells and striated myofibrils in multinucleate myotubes of both lines (Fig. 3). B₉ was negative for myofibril antigen.

Intracellular γ -aminobutyric acid (GABA) and β -alanine are found in cell lines derived from neurectoderm but not in cells derived from mesoderm¹⁶. To determine whether B₉M retained this neurectodermal marker, exponentially growing B₉, B₉M and L6 cells were analysed for free amino acids¹⁶ (Table 1). B₉M and B₉ had very similar free amino acid compositions, but these differed appreciably from L6; B₉M and B₉ contained significant amounts of GABA and β -alanine whereas L6 characteristically lacked these two amino acids. Thus, the phenotypically changed B₉ metabolised amino acids in a manner similar to its parental cell line but distinct from mesoderm-derived skeletal muscle.

Experimental observations in lower vertebrate embryos indicate that certain mesenchymal elements of the head and neck arise from neural crest^{17,18}. By grafting neural crest cells between quail and chick, LeLievre and LeDouarin¹⁸ established that crest cells of the cephalic neural axis down to the 5th somite can give rise to mesenchymal derivatives (including skeletal muscle). If mammalian cephalic neurectoderm can also give rise to mesenchyme, this may explain the rare occurrence of striated muscle elements in primary tumours of the central nervous system (CNS) in man¹⁹, and the derivation of clonal cell lines of skeletal muscle phenotype from tumours induced chemically in developing rat CNS (ref. 5, and D.S., unpublished observation). Rarely, tumours of skeletal muscle arise in the thymus²⁰, which is a pharyngeal pouch derivative. Thymic mesenchyme is predominantly neural-crest derived²¹ and it is likely that its skeletal muscle elements^{22,23} are also of neural crest origin. The thymus is prominently involved in the immunopathology of myasthenia gravis; it is probably the major site of autoimmunisation against AChR which is the cause of failure of neuromuscular transmission in this disease²³. If AChR of thymic myogenic cells and cranial muscle were to share antigenic determinants unique to AChR of neurectodermal origin, the head and neck muscles, which are commonly involved in myasthenia gravis, might be the preferential targets of circulating antibodies with specificities restricted to neurectodermal determinants of AChR. Our observation of skeletal muscle differentiation from a neurectodermal cell line of rat suggests that the cephalic neural crest of mammals, like that of lower vertebrates^{17,18}, might have myogenic potentiality. It remains to be determined whether mammalian neurectoderm contributes to skeletal structures of the head and neck in normal histogenesis.

This work was supported by grants from the Muscular Dystrophy Association (V.L. and D.S.), the NIH (NS 15057, V.L.) and the Kroc Foundation, Santa Ynez, California (V.L.). *Note added in proof:* Striated muscle fibres are known to exist in normal pineal glands, including human²⁴. Recent documentation of the electrophysiological and pharmacological properties of skeletal muscle fibres arising from primary cultures of rat pineals²⁵ further supports our hypothesis of the myogenic potentiality of mammalian neurectoderm.

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The production of the arachidonate metabolite HETE in vascular tissue

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Blood platelets contain two enzyme systems which oxygenate arachidonic acid (AA). First, a cyclooxygenase¹ which metabolises AA into the biologically active prostaglandin endoperoxides (PGG₂ and PGH₂) which are precursors for the platelet prostaglandins PGE₂, PGF_{2 α} and thromboxane A₂ (TxA₂). And second, a lipoxygenase that metabolises AA into 12-L-hydroperoxy-5, 8, 10, 14-eicosatetraenoic acid (HPETE)^{1,2}. HPETE is a labile intermediate which is nonenzymatically reduced in aqueous environments to a more stable metabolite, 12-L-hydroxy-5, 8, 10, 14-eicosatetraenoic acid (HETE). HPETE is produced in significant quantities by aggregated platelets, but its exact biological role is not known. Turner *et al.*³ has suggested that HETE may be a mediator of neutrophil chemotaxis. Vane and coworkers demonstrated that 15-hydroperoxy-5, 8, 10, 14-eicosatetraenoic acid^{4,5} as well as HPETE were potent inhibitors of prostacyclin (PGI₂), the major arachidonate metabolite in vascular tissue. These data suggest that HPETE, if present in vascular tissue, might act as endogenous regulator of PGI₂ production. Using radiochemical techniques and mass spectrometry we have now identified a lipoxygenase enzyme in vascular tissue (rabbit aorta) in which biosynthesis of HETE also occurred. The detection of HETE in this suggests a potential role of the lipoxygenated product as an endogenous regulator of PGI₂ biosynthesis.

Rabbit aorta was cut into small rings (3–5 mg wet weight) and incubated with ¹⁴C-labelled AA at 37°C for 5 min. After incubation, the reaction medium was acidified with 1.0 M formic acid and extracted with ethyl acetate. The radioactive products were partitioned into the organic phase which was then evaporated to dryness under nitrogen. The products were resuspended in ethyl acetate/methanol (1:1) and subjected to TLC.

As shown in Fig. 1a and c, initial incubations of aortic rings yielded an unknown radioactive peak with an R_F value very similar to ricinoleic acid when developed in solvent system I or solvent system II. In a second experiment, indomethacin at a concentration (10⁻⁴ M) which completely inhibits the cyclooxygenase enzyme was added to the incubation medium, and production of this unknown arachidonate metabolite was stimulated by 50% (Fig. 1b, Table 1). From these data it can be concluded that this compound is not a cyclooxygenase product.

AA is prone to nonenzymatic oxidation and this mechanism might account for the unknown component. To differentiate between nonenzymatic and enzymatic oxidation of AA, aortic rings were preincubated with eicosatetraenoic acid (ETYA),

Table 1 Effects of inhibitors on HETE production in rabbit aorta

Inhibitors	HETE (pmol)	% Change from control
—	125.47*	
Indomethacin (1×10^{-4} M)	194.31*	+55
—	107.86†	
ETYA (1×10^{-4} M)	42.08‡	-61
—	149.18‡	
PCMB (2×10^{-3} M)	78.03‡	-48

* An average of three animals.

† An average of two animals.

‡ An average of five animals.

which inhibits both lipoxygenase and cyclooxygenase¹. As shown in Table 1, ETYA (10^{-4} M) completely blocked the formation of this compound, suggesting an enzymatic (lipoxygenase) pathway for the formation of this unknown compound.

If the unknown were HETE, selective inhibition of lipoxygenase would be expected to abolish the formation of this compound and desuppress the biosynthesis of PGI₂. Nugteren⁶ found that para-chloromercuribenzoate (PCMB) was a selective inhibitor of human platelet lipoxygenase at 2×10^{-3} M. In our studies, incubation of PCMB (2×10^{-3} M) with aortic rings (Fig. 1d, Table 1) caused 50% inhibition of formation of the unknown compound, but no stimulation of PGI₂. PCMB tested at various concentrations with lipoxygenase from washed human platelets did not selectively inhibit HETE production—PCMB at 2×10^{-3} M inhibited formation of both HETE and PG (data not shown).

We further characterised the unknown component by gas chromatography-mass spectrometry (GC-MS) analysis. The TLC zone of interest was scraped and eluted with methanol. This extract was concentrated and derivatised with

diazomethane followed by trimethylsilylation¹. The sample was then analysed by selected ion monitoring on a Hewlett Packard model 5985 GC-MS system. Characteristic ions of HETE at *m/e* 391 (M-15), *m/e* 295 (base peak) and *m/e* 225 were observed at the expected retention time. These data show the unknown component to be HETE.

Since the intermediate HPETE is an inhibitor of PGI₂ biosynthesis, the lipoxygenase enzyme in rabbit aorta may have a role in modulating PGI₂ production in vasculature. A high activity of lipoxygenase would suppress PGI₂ formation and result in vasoconstriction, and a low level of lipoxygenase would favour PGI₂ biosynthesis and cause vasodilation. Our findings suggest a physiological role of the lipoxygenase in the cardiovascular system.

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Tumour promoter induces anchorage independence irreversibly

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Tumour-promoting phorbol esters elicit a variety of molecular responses from cells in culture¹⁻³. Phorbol esters are also active as promoters of neoplastic transformation in 10T1/2 mouse fibroblasts, previously initiated by polycyclic aromatic hydrocarbons⁴ or UV⁵ or X-ray irradiation⁶. As many *in vivo* studies of the tumour-promoting activity of phorbol esters have been carried out in mouse skin⁷, it seems desirable to use mouse epidermal cell lines to study the mechanism of tumour promotion *in vitro*. Mouse epidermal cell lines would be particularly useful if they responded to phorbol esters by progressing towards a neoplastic phenotype. We have previously reported the development of cell lines derived from primary mouse epidermal cultures after carcinogen or solvent exposure⁸. Some of these cell lines remained non-tumorigenic for many passages and failed to form colonies in soft agar (frequency less than 10^{-4})⁹. We next asked whether some of these non-tumorigenic cells might respond to tumour promoters like 'initiated' or 'post-initiated' cells *in vivo* by progressing towards a neoplastic state^{9,10}. We report here the identification of three epidermal cell lines which respond to tumour-promoting but not to non-promoting phorbol esters by irreversibly acquiring capacity to grow in soft agar. As anchorage-independent growth characterises malignant cells derived from a variety of sources including mouse epidermis^{8,11}, this response to phorbol esters may be analogous to a late stage of tumour promotion *in vivo*.

Primary epidermal cell cultures¹² were prepared from newborn BALB/c mice⁸ or non-inbred Sencar mice⁹ which had been bred for sensitivity to a 7,12-dimethylbenz (a) anthracene (DMBA)/12-O-tetradecanoyl phorbol-13-acetate (TPA) initiation-promotion scheme of skin carcinogenesis¹³. Cell lines were derived after exposure to carcinogen or to solvent alone as previously described^{8,9}. All of these cell lines retained epidermal-specific cell-surface antigen (N.H.C. *et al.*, in preparation).

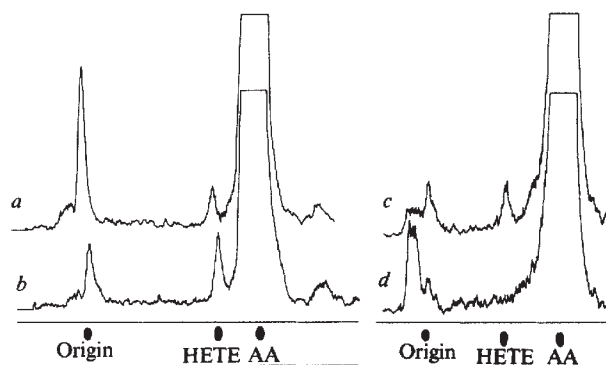


Fig. 1 TLC radiochromatograms of arachidonic acid metabolites in rabbit aorta. Rabbits (2-4 kg) were each given intravenously 1,000 units of sodium heparin to avoid blood clotting and anaesthetised with sodium pentobarbital. The animals were killed and their aortas excised and cut into small rings (4-5 mg per ring) in 50 mM Tris-HCl (pH 7.5) at 4 °C. Each incubation was carried out on 80-90 mg tissue with 7 nmol ¹⁴C-labelled AA and 0.5 ml pH 7.5 Tris buffer in the presence or absence of a drug. The incubation was allowed to proceed for 5 min with shaking at 37 °C in a waterbath. Then the reaction was quenched with 1.0 M formic acid, extracted twice with three times its own volume of ethyl acetate and subjected to TLC. Authentic standards of PG and fatty acid were chromatographed in parallel with unknowns. Zones of radioactivity were detected by a Packard radioscaner and by iodine vapour visualisation. The zones were collected for liquid scintillation counting. a, An incubation in the absence of any drug using TLC system I (chloroform/methanol/acetic acid, 60:40:3). b, An incubation in the presence of 1×10^{-4} M indomethacin using TLC system I. c, An incubation similar to a but using TLC system II (ethyl acetate/2,2,4-trimethylpentane/water, 75:75:100). d, An incubation in the presence of 2×10^{-3} M PCMB using TLC system II.

Table 1 Induction of growth in 0.33% agar medium after exposure to TPA

Cell line	Initial treatment	No. of colonies per 10 ⁴ cells			
		10% Serum		20% Serum	
		Solvent control	TPA	Solvent control	TPA
JB-6	Solvent	<1	696	84	3888
D-2	DMBA	<1	24	84	756
JB-10	MNNG	<1	72	36	264

JB-6 and JB-10 cell lines were derived from solvent or MNNG-treated BALB/c primary cultures as described previously^{8,9}. The D-2 line was derived from DMBA-treated (0.01 $\mu\text{g ml}^{-1}$, twice a week for 3 months) Sencar primary cultures. Cells were grown in monolayer culture in Eagle's minimal essential medium containing 10% fetal calf serum (FCS) and antibiotics and replated once a week⁸. Cells were suspended in 0.33% Difco agar medium⁸ containing solvent alone (0.1% DMSO) or TPA at 0.1 $\mu\text{g ml}^{-1}$ (1.6×10^{-7} M) and layered at 10⁴ cells per 60-mm dish over a 0.5% agar base. The temperature of agar medium when mixed with phorbol esters was less than 40 °C. Assays were carried out in duplicate at both 10% and 20% FCS concentrations without refeeding and colonies were counted at 14 days. Cells were assayed at the following passages: JB-6: 35–51; D-2: 10–24; JB-10: 31–39. Each value is the mean for 3 to 8 separate experiments in which the s.e.m. ranged from 5 to 15% of the value.

While some lines were tumorigenic and formed colonies in 0.33% agar⁸, others failed to grow in agar (in 10% serum) for at least 39 passages^{8,9}. Twenty-one lines of the latter group were screened for colony formation in agar in response to tumour promoters. Three responders were identified including BALB/c lines JB-6, initially exposed only to solvent⁸, and JB-10, initially exposed to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)⁸; and a Sencar line D-2, initially exposed to DMBA (N.C., unpublished). As with all anchorage-dependent mouse epidermal lines tested so far, JB-6 and JB-10 did not give rise to tumours after injection into syngeneic newborns at passages 57 and 15 respectively (refs 8, 9 and unpublished).

When exposed to the tumour-promoting phorbol ester TPA in agar medium, these three non-tumorigenic cell lines showed anchorage-independent growth (Table 1). Colony induction occurred at serum concentrations of 10% and 20%, with greater sensitivity for detection of induced colonies and higher background at 20% serum. This background may reflect weak colony inducing activity of serum growth factor(s) in agar medium. One

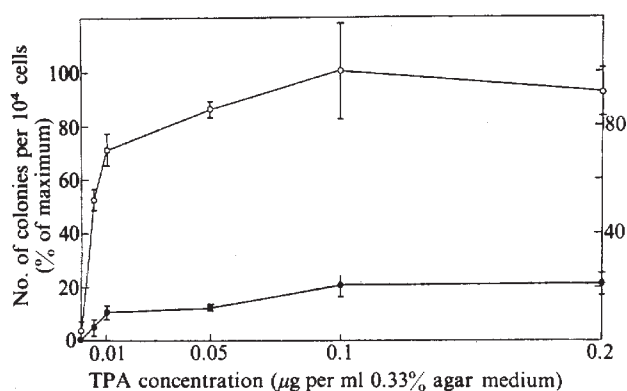


Fig. 1 Effect of TPA concentration on colony formation in soft agar. Assay of growth in 0.33% agar medium was carried out as described in the Table 1 legend. TPA concentration ranged from 0.005 to 0.2 $\mu\text{g ml}^{-1}$ (8×10^{-9} M– 3.3×10^{-7} M). JB-6 cells were assayed at passages 35 to 51. Each point represents the mean of 4 to 16 experiments. Vertical bars indicate s.e.m. Colony forming efficiency is expressed as a per cent of the maximum induced value (that obtained with 0.1 $\mu\text{g ml}^{-1}$ TPA in 20% serum). This form of expression allows the combining of data from a number of experiments as colony forming efficiency varies with serum batch (unpublished). ●, 10% serum; ○, 20% serum.

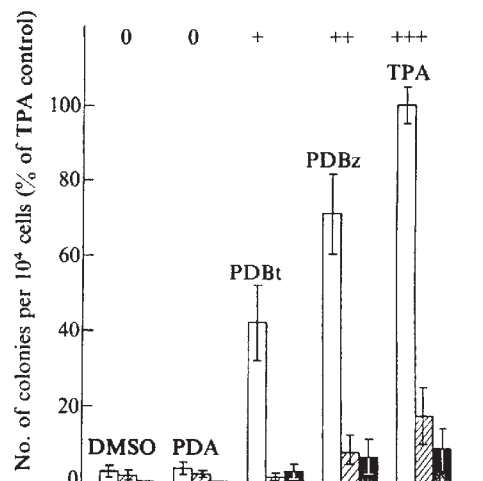


Fig. 2 Correlation of colony inducing activity with tumour-promoting activity of phorbol esters. Assay of growth in 0.33% agar medium was carried out as described in the Table 1 legend. All phorbol esters were used at a concentration of 0.1 $\mu\text{g per ml}$ agar medium and a dimethyl sulphoxide (DMSO) concentration of 0.1%. The rank ordering of tumour-promoting activity of phorbol esters indicated by 0 to +++ is based on Baird and Boutwell¹⁶. Open bars: JB-6, 20% serum. Shaded bars: D-2, 20% serum. Solid bars: JB-6, 10% serum. PDBz, phorbol-12,13-dibenzoate; PDBt, phorbol-12,13-dibutyrate; PDA, phorbol-12,13-diacetate; DMSO, dimethyl sulphoxide control.

growth factor, epidermal growth factor (EGF), has been reported to enhance skin tumour yield in 3-methylcholanthrene-treated mice¹⁴. Recent results (unpublished) have shown that EGF and sarcoma growth factor of Todaro and coworkers¹⁵ will induce growth in agar by JB-6 cells. Previous findings indicated that epidermal cell lines which formed colonies at frequencies less than 1 per 10⁴ in 10% serum in soft agar consistently turned out to be non-tumorigenic^{8,9}. In contrast, tumorigenic lines usually formed colonies at frequencies greater than 200 per 10⁴ in 10% serum. Hence, it seems likely that this TPA-dependent increase in anchorage independence represents induction of tumour cell phenotype. Of the three cell lines, JB-6 showed the highest response to TPA with 39% of the cells giving rise to colonies in 20% serum. To acquire this responsiveness, the JB-6 line required some 30 or more passages in culture. Cells from earlier passage (for example 18, 20, 28) showed no detectable response to TPA in agar (data not shown). Thus whatever 'initiation' or post-initiation events occurred in this solvent-treated line, they did so independently of exposure to added carcinogen and required considerable time in culture. In contrast the TPA responsiveness of D-2 and JB-10 appeared in earlier passages (10 and 15 respectively) and may have resulted from carcinogen exposure during primary culture (see Table 1).

Figure 1 shows the dependence of colony induction on TPA concentration. TPA at concentrations from 0.005 to 0.2 $\mu\text{g ml}^{-1}$ induced colony formation with maximum induction occurring at 0.1 $\mu\text{g ml}^{-1}$ (1.6×10^{-7} M). The dose-response curves are of similar shape in 10% or 20% serum but absolute colony forming efficiency is 4–5-fold higher in 20% serum. TPA at a concentration as low as 10^{-10} M has been found to induce colony formation in JB6 cells grown in agar medium containing 20% serum (data not shown).

Figure 2 indicates that other tumour-promoting phorbol esters but not non-promoting phorbol esters induced anchorage-independent growth in JB-6 and D-2 cells. For JB-6 at 20% or 10% serum the order of colony inducing activity was as follows: TPA > phorbol dibenzoate > phorbol dibutyrate, with the non-promoter phorbol diacetate showing no inducing activity (values identical to solvent control). In D-2 cells only TPA and phorbol dibenzoate induced colony formation. The colony inducing activity paralleled the tumour promoting

activity reported for these phorbol esters by Baird and Boutwell¹⁶.

Many changes induced by tumour-promoting phorbol esters in normal cells are transient and tend to mimic the neoplastic phenotype¹⁷. These changes include loss of cell-surface fibronectin³ and induction of high levels of plasminogen activator¹⁸ and ornithine decarboxylase¹⁹⁻²¹. Many of the phenotypic changes become permanent during pre-neoplastic progression at the time of conversion to overt malignancies often under the influence of promoters²². Thus if the TPA responsive cell lines represent a late stage in tumour promotion, one would expect promoter-induced anchorage independence to be irreversible.

After induction by TPA in agar, colonies were removed with a capillary tube and grown in monolayer culture to yield cell lines, each derived from a single agar colony. The cell lines were then suspended in 0.33% agar medium in the absence of TPA to determine whether the cells retained the capacity for anchorage-independent growth. As shown in Table 2, all of the 11 lines obtained formed colonies in soft agar at frequencies ranging from 96 to 1,992 per 10^4 cells in 10% serum and from 678 to 4,968 per 10^4 cells in 20% serum. Even the lower values were many times higher than those found for the untreated control. The original values obtained for colonies induced in the presence of TPA (TPA-treated control) are presented for comparison. The values for agar derived colonies ranged from 15% to 300% of the original TPA induced values and remained undiminished for at least 2 months following the colony isolation. Cell lines derived from the D-2 line (see Table 1) after induction of anchorage independence by TPA were also found to be stable for growth in soft agar (data not shown). The retained values for the D-2 derived lines were 7-11-fold higher than the original TPA-induced values. Retention of anchorage independence by TPA treated JB10 cells has not yet been tested.

The mechanism of the agar colony response to tumour promoters has yet to be elucidated. Studies in our laboratory (ref. 22, and manuscript in preparation) suggest that TPA

induces a shift in anchorage independence rather than selecting for pre-existing anchorage-independent cells, as colonies form from single JB6 cells (plated one per well in soft agar) in response to TPA. Because induction of anchorage independence by tumour-promoting phorbol esters seems to occur rapidly and irreversibly in the JB-6 and D-2 epidermal cell lines, the process may be analogous to a late phase of tumour promotion *in vivo* in which TPA apparently converts benign pre-malignant lesions to malignant tumours irreversibly^{22,23}.

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Tumour promoters enhance anchorage-independent growth of adenovirus-transformed cells without altering the integration pattern of viral sequences

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Carcinogenesis often involves interaction between multiple factors and proceeds through several discrete steps¹⁻⁸. In mouse skin two distinct phases, 'initiation' and 'promotion', have been described². The most potent tumour-promoting agents in mouse skin are 12-O-tetradecanoylphorbol-13-acetate (TPA) and related diterpenes³⁻⁵. A significant experimental advance in studies on the mechanism of action of tumour promoters has been the demonstration that they induce specific biochemical and biological effects in cell culture⁶⁻⁸. These effects include phenotypic changes that mimic those seen during transformation; modulation of differentiation; enhancement of the expression of markers of transformation in cells that are already transformed; and an increased yield of transformed foci in cultures exposed to chemical carcinogens or radiation⁶⁻¹¹. We have previously reported that TPA also increases the yield of transformed foci in rat embryo cultures previously infected with a mutant of type 5 adenovirus¹². When foci of adenovirus-transformed cells are picked, cloned and serially passaged, they

Table 2 Retention of anchorage independence in the absence of TPA

Cell line	Derivation		No. of colonies per 10^4 cells	
	Cells	Passage	10% Serum	20% Serum
JB6		62-74	7	128
JB6 + TPA (0.01 $\mu\text{g ml}^{-1}$)		62-74	649	2,073
T61	JB6	70	138	1,410
T62	JB6	70	1,992	3,504
T63	JB6	70	96	1,050
T64	JB6	70	824	2,784
T2111	JB6 C121	23	216	678
T2121	JB6 C121	23	852	3,594
T2122	JB6 C121	23	432	1,848
T2123	JB6 C121	23	162	1,632
T2124	JB6 C121	23	756	4,968
T2125	JB6 C121	23	1,050	2,796
T2126	JB6 C121	23	492	2,820

Cells were suspended in 0.33% agar medium as described in the legend to Table 1. Except for the TPA-treated controls (JB6 + TPA), all cell lines were grown in agar in the absence of TPA. Lines T61-T64 were derived from single colonies induced in soft agar after exposure of JB-6 cells to TPA (0.01 $\mu\text{g ml}^{-1}$) in 10% serum. Lines T2111-2126 were obtained from colonies produced after identical exposure of a clone derived (by growth from single cells in standard medium) from the JB-6 line, passage 50 and designated JB6 Clone 21. Cell lines derived from soft agar colonies were grown for 2 passages in monolayer culture (2-3 weeks) before assaying for retention of anchorage independence. Clone 21 yielded values for untreated controls and TPA-treated controls which were about 20% higher than the respective values for JB-6. Cell lines derived from background colonies (without TPA) in 20% serum retained colony forming frequencies similar to the original background levels shown. Each value is the mean for 2 experiments run in duplicate. The differences from earlier passages (Table 1) for solvent and TPA treated JB-6 controls may be attributable to a change in serum batch.

undergo progressive changes, the most notable of which is acquisition of anchorage-independent growth¹³. The present study demonstrates that although TPA enhances the acquisition of anchorage-independent growth in adenovirus-transformed cells (and not in normal cells), this alteration in growth properties is not due to a change in the integration pattern of adenovirus DNA sequences in the genome of the host cell.

Normal secondary rat embryo (2°RE) cultures were infected with a mutant of type 5 adenovirus, H5ts125. This mutant was used because at 37 °C it is defective in DNA replication and has a higher transforming efficiency than wild-type virus¹⁴. In some experiments, the cultures were exposed to chemical carcinogens just before virus infection¹². Epithelioid foci characteristic of adenovirus transformation were picked 5 to 6 weeks later, cloned and then passed in serial culture^{12,13}. The presence of adenovirus DNA sequences in all of the isolated epithelioid clones was demonstrated by reassociation kinetics of ³²P-labelled Ad5 DNA *Hind*III restriction fragments and excess unlabelled transformed cell DNA¹⁵. We found that during early passages (<10) these clones had a low or negligible capacity to grow in agar, whereas at later passages (>30); they spontaneously acquired the ability to grow in agar, although with varying cloning efficiencies¹³.

Although TPA does not induce growth in agar of normal 2°RE cells, it causes an appreciable enhancement of the agar cloning efficiency of the adenovirus-transformed clones (Table 1). With certain early passage adenovirus-transformed clones,

growth in agar was undetectable unless TPA was added. With later passages, these clones spontaneously acquired the ability to grow in agar but their growth in agar was significantly enhanced by TPA. Similar results were obtained with 14 other clones of adenovirus-transformed cells¹³. The E11-E (assayed before 10 serial passages) and E11-L (assayed after 30 serial passages) clones have been studied in greater detail. Concentration curves demonstrated that 3 ng ml⁻¹ was the optimal concentration of TPA (in agar) to effect anchorage-independent growth; a half-maximal effect was obtained with 1 ng ml⁻¹ (1.6 × 10⁻¹⁰ M). Specificity of the TPA effect was demonstrated by the fact that 200 ng ml⁻¹ of either phorbol or 4α phorbol-12-13-didecanoate (4αPDD), which are inactive as tumour promoters on mouse skin³⁻⁵, failed to enhance growth in agar.

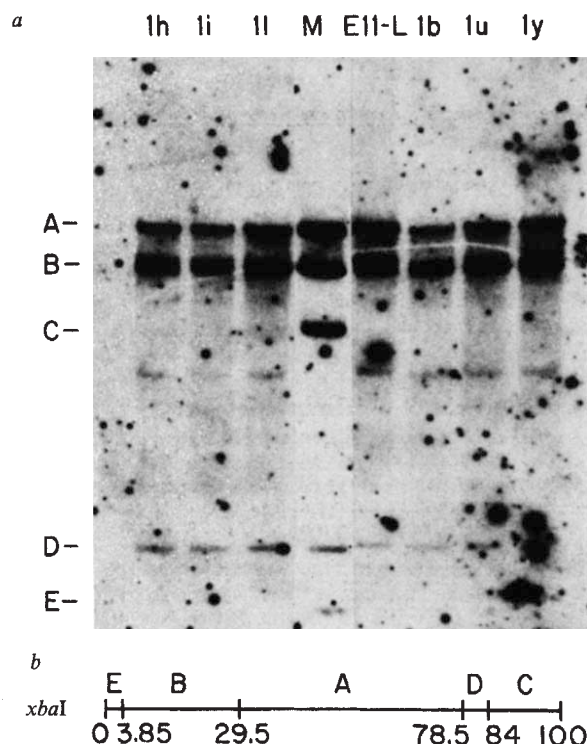


Fig. 1 *a*, Integration pattern of Ad5 DNA in a cloned population of H5ts125-transformed 2°RE cells (E11-L) and in subclones of E11-L isolated from agar either containing or lacking 100 ng ml⁻¹ TPA. Confluent monolayers were grown from each subclone in low Ca²⁺ medium. DNA was isolated as described by Pellicer *et al.*²⁴ and 5 µg of DNA from each clone was cleaved with the restriction endonuclease *Xba*I. The DNA was electrophoresed in 0.7% agarose gels and then transferred to cellulose nitrate sheets according to a procedure slightly modified from the method of Southern²². Ad5 DNA labelled by nick-translation with ³²P to a specific activity of 2–4 × 10⁸ c.p.m. per µg DNA²⁴ was used as a probe for filter hybridisation. The slot labelled M contains an artificial mixture of 5 µg DNA isolated from 2°RE cells and 5 × 10⁻⁵ µg purified Ad5 DNA cleaved with *Xba*I restriction endonuclease. The bands on the autoradiographs are identified as A–E. *b*, *Xba*I restriction map of Ad5 DNA.

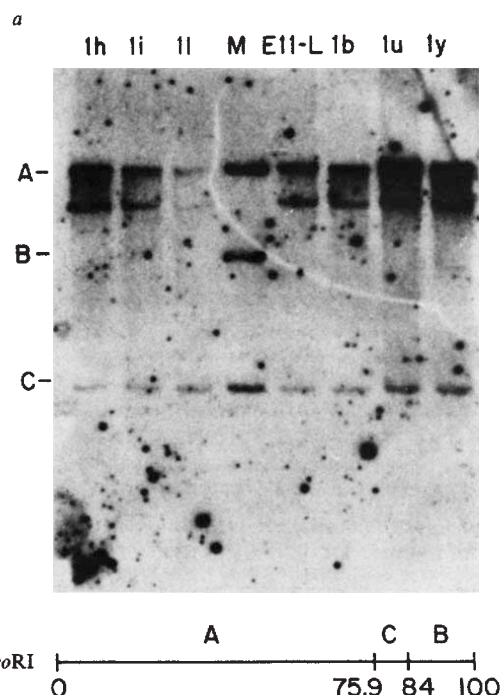


Fig. 2 *a*, Integration pattern of Ad5 DNA in a cloned population of H5ts125-transformed 2°RE cells (E11-L) and in subclones of E11-L. 5 µg of DNA was cleaved with *Eco*RI and hybridised with Ad5 DNA as described in Fig. 1 legend. The varying intensities of the bands in the different subclones from E11-L are due to uneven transfer of the DNA from the gel to the cellulose nitrate filter. The external B fragments migrated considerably more slowly than the corresponding marker B fragment. Note, however, that the external A fragments in transformed cell DNA did not appear to migrate more slowly than the corresponding A fragment of viral DNA because of the poor resolving power of the gel system in the region of the large A fragment (26.6 kilobase pairs). *b*, *Eco*RI restriction map of Ad5 DNA.

To determine whether or not the TPA enhancement of growth in agar was an irreversible effect, subclones of E11-L cells were isolated from agar lacking or containing TPA and further studied. Even in the absence of TPA these subclones grew with a 3.5- to 14.6-fold greater efficiency in agar than the parental clone (Table 1). Note that, with the exception of one subclone (E11-A-11), the addition of TPA to the agar resulted in a further enhancement (1.3- to 4.8-fold) in agar growth, and in some cases the cloning efficiency in agar then exceeded 50% (Table 1). Analysis of 14 additional E11-L subclones isolated from agar, which contained or lacked TPA, revealed that with serial passage in the absence of TPA none of these subclones reverted to the lower agar cloning efficiency characteristic of the parental E11-L clone. As with the parental E11-L clone, growth in agar of the subclones was not enhanced by 200 ng ml⁻¹ of the non-tumour-promoting agents phorbol or 4αPDD.

When studied in monolayer culture, E11-L and all of its subclones had shorter average doubling times and higher saturation densities than normal 2°RE cells (Table 2). TPA did not significantly alter the average doubling time of normal 2°RE or any of the adenovirus-transformed clones (Table 2). TPA did, however, increase the saturation density of some, but not all of these cells. With serial passage E11 cells acquired a higher saturation density (compare E11-L to E11-E, Table 2) and, in general, the E11-L subclones derived from agar had higher saturation densities than the parental E11-L clone. However, there was no direct relationship between saturation densities and cloning efficiencies in agar of E11-L or its subclones, either in the absence or presence of TPA.

Previous studies have demonstrated that the transformation of Syrian hamster embryo cells following a single exposure to the carcinogen benzo(a)pyrene involves a series of progressive changes—morphological transformation, enhanced fibrinolytic activity, growth in agar and tumorigenicity^{16,17}. These are acquired in a temporal fashion during repeated serial passage of the culture^{16,17}. The acquisition of anchorage independence and tumorigenicity following the exposure of guinea pig embryo fibroblasts¹⁸, rat brain cells¹⁹, rat liver cells²⁰, or mouse epidermal cells²¹, to a single exposure with chemical carcinogens has also been found to require extended serial passage (in these cases several months) in culture.

entire Ad5 genome. Furthermore, the Southern blotting technique revealed that the integration patterns of E11-E and E11-L were identical (data not shown) and that when the transformed cell DNA was cleaved with *Xba*I endonuclease the integration patterns of viral DNA in E11-L and its subclones were indistinguishable (Fig. 1). Note that because of their attachment to host DNA, the terminal C and E fragments migrated more slowly than the C and E viral DNA marker fragments. Moreover, the C fragments of E11-L and all of its subclones co-migrated; similarly, all E fragments from E11-L and its subclones isolated from agar with or without TPA co-migrated with each other. In contrast, the internal fragments (A, B and D) from all clones co-migrated with the corresponding internal fragments of marker viral DNA, demonstrating that the viral DNA was integrated as intact linear molecules without interruption by cellular sequences and hence without disturbed specific endonuclease restriction sites. Similar conclusions can be drawn from patterns found following cleavage of transformed cell DNA with the *Eco*RI restriction endonuclease (Fig. 2).

The data presented here indicate that acquisition of anchorage-independent growth by adenovirus-transformed cells requires prolonged serial passage and that exposure to TPA markedly accelerates the acquisition of this property. These events are not due simply to random mutation and cell selection; their frequency is too high and they do not obey the properties of

Table 1 Effect of TPA on anchorage-independent growth of normal and adenovirus-transformed rat embryo cells

Cell type	Colony number		Agar cloning efficiency (%)		TPA enhancement factor
	-TPA	+TPA	-TPA	+TPA	
2°RE	0	0	<0.001	<0.001	0
A18-E (<10 pass.)	0	2.4 ± 0.8	<0.001	0.1	>100
A18-L (>30 pass.)	2.8 ± 0.4	10.4 ± 2.2	0.1	0.5	5.0
E7-E (<10 pass.)	0	4.8 ± 2.0	<0.001	0.2	>200
E7-L (>30 pass.)	23.2 ± 2.4	54.4 ± 5.0	1.2	2.7	2.3
E11-E (<10 pass.)	22.2 ± 5.8	58.4 ± 6.4	1.1	2.9	2.6
E11-L (>30 pass.)	59.2 ± 17.8	232.0 ± 18.4	3.0	11.6	3.9
Subclones of E11-L isolated from agar					
E11 A-1 1h	252 ± 30.4	956 ± 76.8	12.6	47.8	3.8
E11 A-1 1i	204 ± 36.4	988 ± 59.2	10.2	49.4	4.8
E11 A-1 1l	424 ± 30.4	434 ± 40.6	21.3	21.7	1.0
Subclones of E11-L isolated from agar plus TPA					
E11 A-T-1 1b	826 ± 68.6	1,118 ± 72.4	43.1	55.9	1.3
E11 A-T-1 1u	232 ± 17.0	1,034 ± 72.2	11.6	51.7	4.5
E11 A-T-1 1y	245 ± 16.4	1,102 ± 96.6	12.7	55.1	4.3

Secondary rat embryo (2°RE) cultures were established from 14-d gestation Sprague-Dawley rat embryos²³ and were grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Clonal populations of type 5 adenovirus H5ts125-transformed 2°RE cells were formed as previously described¹² and were grown at 37 °C in 0.1 mM Ca²⁺ medium supplemented with 7.5% FBS (low-Ca²⁺ medium)²³. Clones A18 and E7 were originally isolated from two foci (on different plates) of acetone pretreated (solvent control) H5ts125-transformed 2°RE cells and clone E11 cells were derived from a focus of benzo(a)pyrene (0.05 µg ml⁻¹)-pretreated, H5ts125-transformed 2°RE cells^{12,13}. The original transformed cell population was cloned and tested for growth in agar following early (E-, <10) and late (L-, >30) serial passage. E11-L subclones were isolated directly from agar lacking or containing 100 ng ml⁻¹ TPA and were maintained as separate cell lines in low Ca²⁺ medium. Agar cloning efficiency of early (<10) and late (>30) passage adenovirus-transformed clones and E11-L subclones was determined by plating 2 × 10³ cells in 4 ml of agar overlay medium containing 0.4% Noble agar prepared in low Ca²⁺ medium on top of prehardened 4-ml base layers consisting of 0.8% Noble agar in the same medium. Parallel cultures contained TPA at a final concentration of 100 ng ml⁻¹ incorporated in both the agar overlay and base layers. Cultures were fed after 4 and 10 days with 3 ml of 0.4% Noble agar in low-Ca²⁺ medium containing or lacking 100 ng ml⁻¹ TPA. Agar cloning efficiency of 2°RE cells in the presence or absence of 100 ng ml⁻¹ TPA was determined as above except 2 × 10⁴, 2 × 10⁵ or 2 × 10⁶ cells were seeded and the medium was DMEM in Noble agar. Colony number was determined by light microscopy after 14 days (colony size >0.1 mm) and 21 days (colony size >2 mm). Results reported are the average colony number from four replicate plates ± s.e.m. TPA enhancement factor refers to agar colony-forming ability in the presence of TPA relative to cultures not receiving TPA. Phorbol and 4α-phorbol-12-13-didecanoate (4αPDD), two compounds inactive in tumour promotion, did not inhibit agar growth or induce enhanced agar cloning efficiency of any of the adenovirus-transformed clones even when tested at 200 ng ml⁻¹.

Experiments were therefore carried out to determine whether acquisition of anchorage independence following serial passage of adenovirus-transformed cells, or TPA treatment in agar, altered the amount or the pattern of viral DNA integration. Nucleic acid hybridisation techniques, reassociation kinetics¹⁵, and Southern blotting filter hybridisation²², were used to determine the amount of DNA present and the state of the DNA (that is, whether still integrated as single linear molecules). Reassociation kinetics indicated that E11-E and E11-L transformed cells each contained approximately one copy of the

a classical mutation in a fluctuation analysis (P.B.F. and I.B.W., unpublished). In addition, the data presented indicate that these changes in cell phenotype are not due to alterations in the extent or state of integration of adenovirus sequences in the genome of the host cell. Two major alternatives remain to be considered: (1) a change in expression of the integrated viral sequences, either at the level of transcription and RNA processing or protein synthesis; or (2) alterations in the expression of specific host genes which might act in concert with integrated viral genes to modulate the phenotype of the transformants. We are now

Table 2 Effect of TPA on doubling time and saturation density of normal and adenovirus-transformed rat embryo cells

Cell type	Average population doubling		Saturation density (cells cm ² × 10 ⁵)	
	-TPA	+TPA	-TPA	+TPA
2 ⁰ RE	32.2 ± 1.2	31.6 ± 1.5	1.7	2.1
A18-E (<10 pass.)	32.5 ± 1.0	29.5 ± 1.0	6.7	8.2
A18-L (>30 pass.)	19.4 ± 2.1	20.2 ± 1.7	8.7	9.9
E7-E (<10 pass.)	46.3 ± 3.5	45.7 ± 2.8	5.3	6.3
E7-L (>30 pass.)	21.4 ± 1.7	20.2 ± 2.1	7.9	9.5
E11-E (<10 pass.)	18.2 ± 1.0	15.4 ± 0.7	9.2	11.2
E11-L (>30 pass.)	15.2 ± 0.5	15.4 ± 0.7	12.8	14.8
Subclones of E11-L isolated from agar				
E11 A-1 1h	16.1 ± 0.8	14.2 ± 0.3	20.2	24.2
E11 A-1 1i	15.0 ± 0.5	15.5 ± 0.1	18.3	19.6
E11 A-1 1l	16.3 ± 0.4	16.5 ± 0.7	17.2	17.5
Subclones of E11-L isolated from agar plus TPA				
E11 A-T-1 1b	14.2 ± 0.6	14.6 ± 0.2	15.0	15.8
E11 A-T-1 1u	15.4 ± 0.7	13.0 ± 0.4	19.4	25.2
E11 A-T-1 1y	14.8 ± 0.8	15.9 ± 1.0	16.9	22.6

The average population doubling times and saturation densities of 2⁰RE and H5ts125-transformed clones in the presence or absence of TPA were obtained by plating 5 × 10⁴ cells per 50 mm tissue culture plate in low-Ca²⁺ medium at 37 °C, changing the media after 6 or 24 h to low-Ca²⁺ medium with or without 100 ng ml⁻¹ TPA and determining cell numbers over a 21-d period. Cells in triplicate plates were dispersed with trypsin/EDTA (0.25% trypsin w/v, 0.2% EDTA w/v) and counted every 24 h using a Coulter counter (Model Zf). The media were changed every 72 h with or without 100 ng ml⁻¹ TPA. Population doubling times were calculated over a 72-h period corresponding to the logarithmic phase of growth. Saturation densities represent the maximum cell densities obtained in confluent cultures grown in the presence or absence of TPA.

analysing transcription patterns of viral mRNA in serially cultured clones of adenovirus-transformed cells, in the presence or absence of TPA, to determine whether changes in the type or amount of specific mRNA transcripts can be detected during the course of progression of these cell cultures. Thus, the cell system described here may be useful for understanding the mechanism by which transformed cells acquire anchorage-independent growth; it may also provide a useful model for studying the complex phenomena involved in tumour promotion and progression.

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Lysozyme-induced T-suppressor cells and antibodies have a predominant idiotypic

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The existence of shared idiotypic determinants on the surfaces of T and B cells is now firmly established, suggesting that on both these cell types immunoglobulin variable regions are expressed which presumably function as antigen receptors. In most systems this has been inferred through the use of anti-idiotypic antibody instead of antigen to induce either helper or suppressor T cells^{1,2}. Recent evidence demonstrates that antigen-specific suppressor or helper factors can also bear idiotypic determinants^{3,4}. It is possible that these factors represent released receptors or portions of receptors. We show here the direct elimination of an antigen-induced T-suppressor population by an anti-idiotypic serum and complement. These suppressor T cells as well as the idiotypic population used to generate the antiserum are each specific for the same limited portion of the multi-determinant antigen, lysozyme. Apparently, these suppressor cells are restricted in specificity as well as share idiotypic with antibodies of the same specificity.

Lysozymes are small (molecular weight ~14,300) globular proteins. The amino acid sequence and three-dimensional structure for lysozymes from many different species are known⁵. The murine response to a series of bird egg white lysozymes, typified by chicken lysozyme (HEL), is under H-2-linked genetic control. Mice of the H-2^b and H-2^s haplotypes (B10 and B10.S) are non-responders while mice bearing other haplotypes are responders⁶. We have demonstrated T-suppressor cells in

Table 1 Anti-idiotypic and complement treatment eliminates the activity of HEL-induced suppressor cells from B10 mice

Spleen cells (×10 ⁶) per culture from mice primed with:		Treatment of HEL-primed spleen cells	Direct PFC per culture on day 4	
CFA	HEL-CFA		HEL	SRBC
2	—	—	231 ± 46	1,326 ± 37
—	2	—	65 ± 20	1,956 ± 449
1.8	0.2	—	53 ± 37	1,233 ± 203
1.8	0.2	NGPS + C'	31 ± 1	1,310 ± 70
1.8	0.2	Anti-id + C'	207 ± 10	1,233 ± 240
1.8	0.2	Anti-id	75 ± 5	1,533 ± 433

In vitro cell cultures were carried out using a miniaturised two-chamber diffusion culture system¹⁴. Culture medium was RPMI 1640 (GIBCO) supplemented with 10% fetal calf serum (Flow), 2 mM L-glutamine, 5 × 10⁻⁵ M 2-mercaptoethanol. B10 mice were primed intraperitoneally with CFA or HEL-CFA (100 µg per mouse) 4 weeks before culture. Spleen cells (2 × 10⁶) were cultured together with 2 × 10⁶ HEL-SRBC in 0.1 ml in the inner chamber, separated by a dialysis membrane from the reservoir in which 1.5 ml of medium was placed. At day 4 of culture, cells were collected and tested for direct PFC formation against HEL-burro (B)RBC, BRBC or sheep(S)RBC using the Cunningham and Szenberg technique¹⁵. Antiserum treatment: before culturing, 2 × 10⁶ spleen cells were pelleted and resuspended in 0.1 ml of heat-inactivated normal guinea pig serum (NGPS) (final dilution 1:10) or guinea pig anti-idiotypic (anti-id) (1:5) for 30 min at 4 °C. Cells were then washed once, resuspended in 0.1 ml rabbit complement (Cedarlane Low-Tox), diluted 1:8, incubated at 37 °C for 30 min and finally washed three times before culture. PFC data are presented as arithmetic means ± s.e.m. from three replicate cultures.

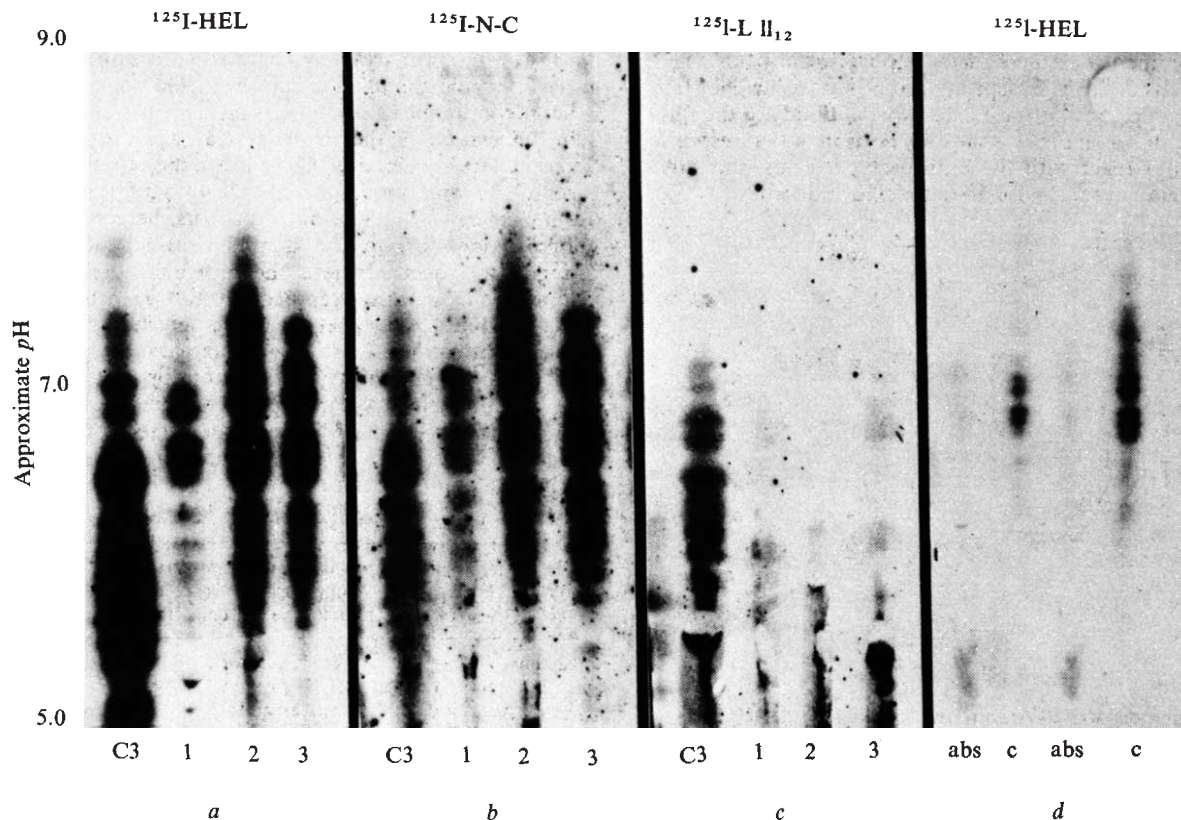


Fig. 1 Autoradiographs of isoelectric focusing in polyacrylamide gels showing specificity and idiotype of anti-HEL antibodies. *a*, IEF patterns of 2 μ l A/J C3 and B10.A (1, 2, 3) immune ascitic fluids, stained with 125 I-HEL. *b*, Same fluids as in *a* stained with 125 I-N-C peptide. *c*, Same fluids as in *a* stained with 125 I-L_{II} peptide. Isoelectric focusing was carried out as described previously¹¹. Iodination of N-C peptide, which lacks tyrosine, was accomplished by first reacting the peptide with methyl-*p*-hydroxybenzimidate as described by Wood *et al.*¹², followed by iodination with the Chloramine T method¹³. *d* Represents the effect of absorbing the anti-HEL from B10.A mice 1 and 3 with anti-idiotypic before isoelectric focusing. Briefly, 2 μ l of each B10.A anti-HEL was placed in a small conical centrifuge tube to which 10 μ l guinea pig anti-idiotypic was added. Control tubes were 2 μ l ascitic fluid plus 10 μ l normal guinea pig serum. All tubes were incubated for 30 min at 37 °C and overnight at 4 °C. The entire contents of each tube was then placed on isoelectric focusing gels. If immune complexes form during this incubation, specific antibody bands are eliminated from the autoradiograph, as these complexes appear to be too large to enter the gel. abs, absorption with anti-idiotypic; C, the mixture with normal guinea pig serum. Left two lanes are from B10.A mouse 1; right two lanes are from B10.A mouse 3.

the spleens of B10 mice injected intraperitoneally with HEL emulsified in Freund's complete adjuvant (CFA). Helper cells are demonstrable in the spleens of similarly immunised congenic B10.A mice⁷. We have also established that a disulphide-containing peptide derived from this multi-determinant antigen (N-C peptide = 1-17: Cys 6-Cys 127:120-129) is equally capable of inducing these suppressor cells while another peptide from HEL (L_{II} peptide = 13-105) induces helper T cells in B10 mice⁸. To further characterise T-cell receptors in this system we have examined the effect of anti-idiotypic on HEL-induced suppressor cells from non-responding B10 mice and HEL-induced helper cells from responding B10.A mice.

To investigate the lysozyme T-cell idiotypic repertoire in B10.A and B10 mice, anti-idiotypic antisera were generated against restricted anti-HEL antibody populations, which are often found in B10.A mice. Figure 1*a* shows the isoelectric focusing patterns (IEF) of antibodies from immune ascitic fluid of three B10.A mice after immunisation with HEL. These patterns are more restricted than those produced by similarly immunised A/J mice.

Two lysozyme-derived peptides, N-C and L_{II}, were used to investigate the specificity of these anti-HEL antibodies. Figure 1*b* and *c* show the IEF patterns of the same fluids as in Fig. 1*a* stained with the N-C peptide and the L_{II} peptide, respectively. As can be seen, all the antibody in the ascitic fluid of the HEL-immunised B10.A mice appears to react with the N-C

peptide but not with the L_{II} peptide. Experiments with N-C immunoadsorbents corroborate the N-C staining results (data not shown). The selected A/J fluid shows some L_{II} reactivity but it does not represent a large portion of the total antibody (~15%).

Purified antibody from the ascitic fluid of one B10.A mouse (1 of Fig. 1) was isolated by adsorption to and elution from an HEL immunoadsorbent. The isolated antibodies were used to immunise guinea pigs and the resulting antiserum repeatedly passed over normal B10.A Ig columns. Figure 1*d* shows that the absorbed serum reacts with all the antibody bands present in the immunising population as well as all other B10.A anti-HEL antisera. The addition of large amounts of normal Ig to absorption mixtures had no effect on the ability of the guinea pig anti-idiotypic to absorb HEL-specific antibody bands (data not shown).

As idiotypic determinants are antigenic structures close to or including the antigen-binding site of the antibody molecule, anti-idiotypic antibodies may interfere with the interaction of idiotype with antigen. Figure 2 demonstrates that the anti-idiotypic serum can inhibit the binding of B10.A anti-HEL to radiolabelled HEL by as much as 80%. The same anti-idiotypic has no effect on the binding of B10.A anti-human lysozyme (HUL) antibodies to radiolabelled HUL. This inhibition of HEL binding is consistent with recognition of determinants associated with, or near, the antigen-binding site by the anti-idiotypic serum.

This anti-idiotypic serum reacts with almost all anti-HEL antibodies we have examined regardless of the mouse strain from which they were derived. When L_{II} -specific antibodies occur, they lack the idiotype; in particular the L_{II} -reactive antibody in the fluid from A/J C3 shown in Fig. 1 is the only portion of that response which does not react with the anti-idiotypic. Thus, this anti-idiotypic appears to react with N-C-specific antibodies.

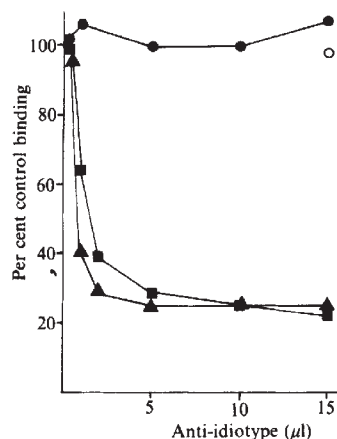


Fig. 2 Inhibition of B10.A anti-HEL binding to ^{125}I -HEL by anti-idiotypic. Inhibition curves obtained when increasing amounts of anti-idiotypic antiserum were used to inhibit the binding of B10.A anti-HEL to ^{125}I -HEL or B10.A anti-HUL to ^{125}I -HUL. Increasing amounts of anti-idiotypic antiserum were added to tubes containing a suitable dilution of anti-HEL ascitic fluid. This dilution was capable of binding 49–55% of 10 ng of ^{125}I -HEL, or 10 ng of ^{125}I -HUL. Following appropriate incubation all the γ -globulin in each tube was precipitated by adding an equal volume of 36% (w/v) Na_2SO_4 . After 2 h incubation at room temperature, the precipitates were collected by centrifugation, washed twice and counted in a Nuclear Chicago γ -counter. Data are presented as the per cent of control binding (that is, binding obtained in reaction mixtures without inhibitor). ■, B10.A mouse 1 anti-HEL; ▲, B10.A mouse 3 anti-HEL; ●, a single B10.A anti-HUL serum binding to ^{125}I -HUL; ○, Inhibition of B10.A mouse 1 anti-HEL binding to ^{125}I -HEL by 15 μl normal guinea pig serum.

This anti-idiotypic serum was used to examine the expression of idiotype determinants on functional T-cell subpopulations. Results in Table 1 demonstrate that preimmunisation of B10 mice with HEL generates a cell population capable of suppressing the anti-HEL plaque-forming cell (PFC) response of primary spleen cell cultures stimulated *in vitro* with HEL-red blood cell (RBC). These suppressor cells have been shown to be sensitive to rabbit anti-mouse thymocyte or anti-I-J treatment⁷. Furthermore, suppressive activity is found in the cell population remaining after passage through nylon wool (B. Araneo, personal communication). Sequential treatment of HEL-primed spleen cells with anti-idiotypic and complement eliminates the ability of these cells to suppress the anti-HEL PFC response, while treatment with normal guinea pig serum and complement does not affect suppression. If complement is omitted and the cells are only incubated with anti-idiotypic for 30 min at 4 °C, attempting to block suppressive activity, almost no effect is observed. These results indicate that the suppressor T cells raised against HEL express some or all of this set of idiotype determinants.

Using the same approach we tried to determine if these idiotypes were expressed on helper T cells. Results shown in Table 2 demonstrate that the helper activity induced in B10.A mice by HEL priming is not affected by treatment of HEL-primed spleen cells with anti-idiotypic and complement, while in the same experiment, HEL-induced suppressor cells in B10 mice are sensitive to this treatment. Even when sodium azide (10^{-2} M) was added to stabilise membrane receptors, the helper

activity was unaffected. This insensitivity may reflect a lower density or different arrangement of receptors on helper cells, or perhaps a rapid shedding of receptor from these cells.

Some preliminary evidence suggests that the helper cells being measured in the present assay are directed against a different determinant on HEL than are the suppressor T cells or B cells. This would be expected to lead to different idiotypic for the helper cells. In some other systems, helper cells can bear idiotypes identical to the dominant B-cell idiotype. Interestingly, this occurs where the antigenic element is multivalent, such as with streptococcal carbohydrates⁹ or phosphorylcholine¹⁰, where helper T cells specific for the repeating determinant can present the same antigenic determinant to B cells. In our system, this type of relationship is obviated because HEL is monovalent for any given epitope. The clear requirement for helper T cells of different specificity from that of the B cell could quite naturally lead to a functional helper population lacking the dominant idiotype.

Table 2 Anti-idiotypic and complement treatment eliminates the activity of HEL-induced suppressor cells from B10 mice but not the activity of HEL-induced helper cells from B10.A mice*

Spleen cells ($\times 10^6$) per culture from mice primed with:	CFA	HEL-CFA	Treatment of HEL-primed spleen cells	Direct PFC per culture on day 4	
				B10	B10.A
2	—	—	—	170 $\pm$ 27	26 $\pm$ 1†
—	2	—	—	11 $\pm$ 7	235 $\pm$ 59
1.9	0.1	—	—	25 $\pm$ 2	155 $\pm$ 5
1.9	0.1	—	NGPS + C'	31 $\pm$ 13	150 $\pm$ 20
1.9	0.1	—	Anti-id + C'	186 $\pm$ 31	161 $\pm$ 29

* Immunisations, *in vitro* cultures, and antisera treatments as described in Table 1. HEL-human RBC were substituted for HEL-SRBC as antigen *in vitro*.

† Primary anti-HEL PFC responses obtained with normal B10.A spleen cells are routinely much lower than those obtained with normal B10 spleen cells.

Thus, the antibody specificity in the secondary response to lysozyme, a multi-determinant antigen, is restricted. Likewise, suppressor cells raised in the non-responder mouse show a remarkably similar restriction to this same determinant. We consider it no accident that receptors on each of these two cell populations should bear the same idiotype determinants. This arrangement ensures that there will be mediation between suppressor T cells and B cells by helper T cells with complementary idiotype or epitypic receptors. We are now studying the rules of antigenic and idiotype circuitry which are involved in the selection of particular B cells for activation.

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Immunoreactive forms of human erythrocyte ankyrin are present in diverse cells and tissues

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Ankyrin is a polypeptide of molecular weight (MW) 200,000 which is tightly bound to the cytoplasmic surface of the human erythrocyte membrane and has been identified as the high-affinity membrane attachment protein for spectrin¹⁻³. This protein has also been shown to be associated with band 3 (ref. 4), the major transmembrane protein in erythrocytes. Ankyrin is thus an example of a polypeptide which links a cytoplasmic structural protein to an integral membrane protein. A water-soluble, 72,000-MW, proteolytic fragment of ankyrin has been purified which retains the ability to bind to spectrin, and competitively inhibits reassociation of spectrin with membranes⁵. Monospecific antibodies directed against this fragment have been prepared¹ and demonstrated to cross-react only with ankyrin among the erythrocyte membrane proteins^{1,4}. The present study reports the use of these antibodies to develop a radioimmunoassay capable of detecting femtomolar quantities of ankyrin, and demonstrates the presence of small but significant amounts of immunoreactivity in a variety of types of cells and tissues.

Ankyrin itself is not suitable as a ligand for radioimmunoassay because it is very sensitive to proteolysis, and has hydrophobic properties which would make nonspecific binding a serious problem¹. The 72,000-MW water-soluble fragment of ankyrin, however, was readily radioiodinated and exhibited low background radioactivity in assay conditions. Monospecific anti-fragment IgG bound ¹²⁵I-labelled fragment with both a high-affinity interaction ($K_D = 0.3$ nM) and a lower-affinity association ($K_D = 12$ nM); 75–80% of the ¹²⁵I-labelled fragment was immunoreactive.

Binding of ¹²⁵I-labelled fragment to low concentrations of monospecific antibody (0.2 nM) was blocked completely by 0.4 pmol of unlabelled fragment, and a significant inhibition occurred with as little as 2 fmol of fragment (Fig. 1). Binding assays were carried out in the presence of Triton X-100, which permitted analysis of membranes and whole cells without prior solubilisation. Human erythrocyte ghosts block binding of ¹²⁵I-labelled fragment completely above 500 ng of membrane protein, and by 50% at 20 ng of protein (Fig. 1).

To estimate the amount of ankyrin present in ghost membranes, it was necessary to control for the possibility that the protein in a native conformation might associate with antibody to a different degree from its association with the soluble fragment. Accordingly, SDS-denatured fragment and ghost membranes were assayed for inhibition of binding. SDS-treated fragment exhibited unchanged activity, whereas SDS-treated membranes were twofold less effective (Fig. 1). The SDS-treated ghosts contained the equivalent of 0.36 pmol or 26 ng of fragment per μ g membrane protein, which agrees closely with the previous estimate that the fragment constitutes 2.4% of the membrane protein⁵. Ankyrin thus comprises 7.2% of the total protein, a value corresponding to about 120,000 molecules per erythrocyte using the data of Steck for erythrocyte membrane proteins⁹. (This value is based on the assumption that the 72,000-MW fragment of ankyrin does not contain antigenic determinants that are repeated in the remainder of the molecule.)

Radioimmunoassay provides quantitative information only when the ligand used as a standard and the material to be

assayed bind to the antibody with the same affinity and the same extent of cross-reaction. However, useful qualitative comparisons of levels of immunoreactivity can be made in heterologous systems, although the actual amount of antigen will in most cases be underestimated. Peripheral human blood neutrophils, platelets and human cultured fibroblasts from foreskin or embryonic lung exhibited a significant amount of immunoreactivity (Fig. 2). ¹²⁵I-labelled fragment was displaced by 50% using 5×10^4 erythrocytes, 8×10^3 neutrophils, 2×10^6 platelets and 1.4×10^5 fibroblasts per assay. These numbers correspond to 6×10^5 copies of ankyrin per neutrophil, 3×10^3 per platelet and about 3×10^4 per fibroblast. The slopes of the inhibition curves of these cells are less than that of erythrocytes, indicating partial cross-reactivity and/or decreased affinity for the antibody. The values for molecules per cell are thus probably underestimates. The activity of these cells is unlikely to be due to proteolysis by lysosomal enzymes, as samples boiled in SDS still compete for binding. Furthermore, problems of nonspecific interference in the assay were minimised by the very small quantities of cells used.

It was important to determine whether immunoreactivity could be detected in various tissues. Due to difficulty in obtaining fresh human material, erythrocytes from other species were compared for cross-reactivity. Rat cells were about 10-fold less active than those of humans, but were 3–5-fold more reactive than cells of horses or rabbits. Membrane fractions from rat brain, liver, kidney, testes and isolated epididymal fat cells all exhibited immunoreactivity, with 50% inhibition at 0.4 μ g for rat erythrocytes, 5.7 μ g for brain, 9.5 μ g for testes and about 12.6 μ g for liver, kidney and fat cells (Fig. 3). These values correspond to the presence of ankyrin at 0.5% of the total membrane protein in brain, 0.2% in liver, kidney and fat cells, and 0.3% in testes, assuming that rat and human erythrocytes

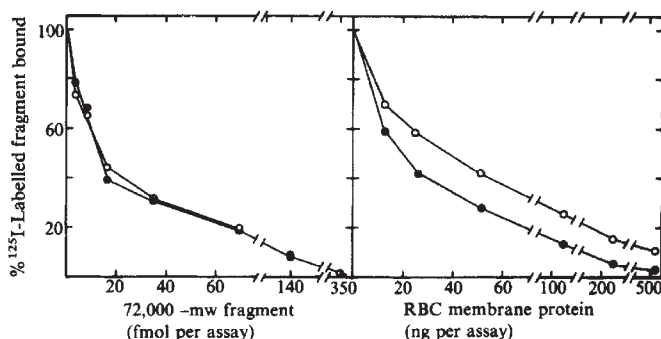


Fig. 1 Inhibition of binding of ¹²⁵I-labelled 72,000-MW fragment to monospecific anti-fragment IgG by unlabelled 72,000-MW fragment (left) and human erythrocyte membrane protein (right). Anti-fragment IgG (0.18 nM) was incubated for 1 h at 0 °C in 0.075 ml of buffer (100 mM NaCl, 10 mM sodium phosphate, pH 7.5, 1 mM EDTA, 250 μ g ml⁻¹ bovine serum albumin, 5 μ g ml⁻¹ pancreatic trypsin inhibitor, 0.2% (v/v) Triton X-100, 10 μ g ml⁻¹ preimmune IgG) in the presence of various concentrations of 72,000-MW fragment or erythrocyte ghost protein which were either untreated (●) or had been exposed to SDS (○). The final concentration of SDS was not more than 0.0025% at the highest dilution of these samples. ¹²⁵I-labelled fragment (prepared by the method of Hunter and Greenwood⁶; 3.6×10^6 c.p.m. pmol⁻¹, 0.4 nM final concentration) and 0.2 μ l protein A-bearing staphylococci were then added as a second antibody⁷, and the incubation was continued for another hour at 0 °C. The reaction was terminated by addition of 3.5 ml 2 M urea, 0.1 M glycine and 1% (v/v) Triton-X-100, and the bacteria, with adsorbed IgG, were collected by centrifugation (10 min, 3,000g), and analysed for ¹²⁵I. The data are presented as per cent of binding in the absence of additions, and are corrected for nonspecific binding by subtracting the value obtained with preimmune IgG alone at each concentration of fragment and membrane protein. Erythrocyte ghosts were prepared by hypotonic lysis in 7.5 mM sodium phosphate, pH 7.5 (ref. 8).

contain the same amount of ankyrin and that the immunoreactive protein has the same molecular weight as ankyrin. Liver homogenates were fractionated by differential centrifugation into microsomal membranes which contained 55% of the immunoreactivity, a mitochondrial/lysosomal pellet which had 7% and a soluble fraction which had 30%. The soluble immunoreactivity may be due to proteolytic cleavage of the membrane-bound protein, for the 72,000-MW fragment of

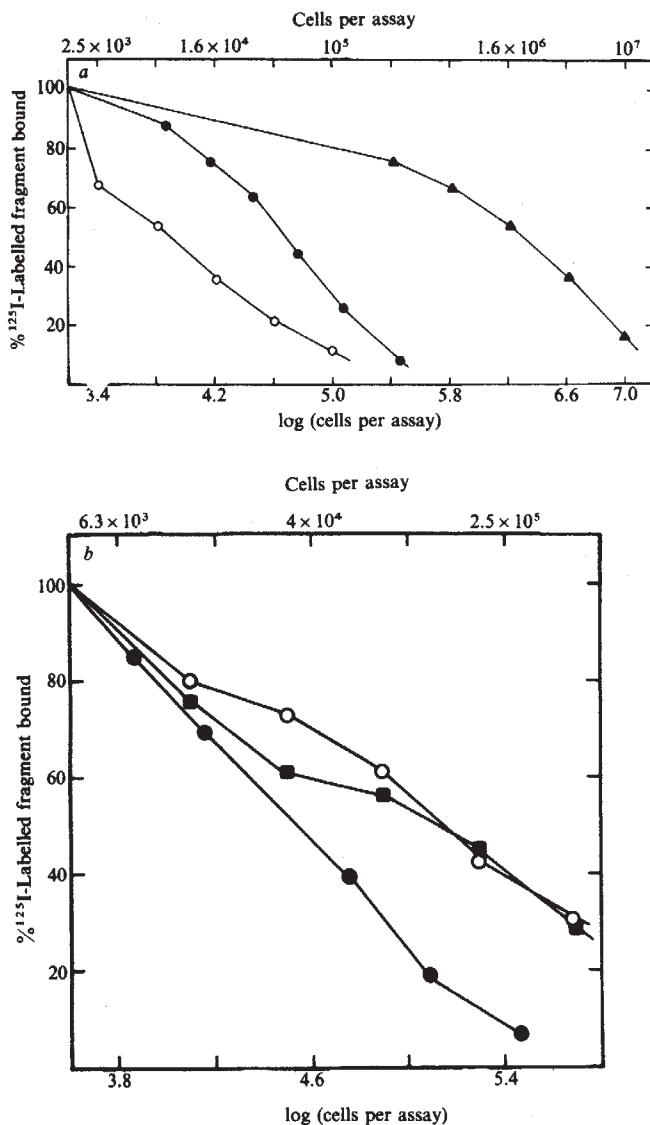


Fig. 2 Inhibition of binding of ^{125}I -labelled 72,000-MW fragment to monospecific anti-fragment IgG by human erythrocytes, neutrophils and platelets (a), and by cultured human foreskin fibroblasts and Wi-38 lung fibroblasts (b). Anti-fragment IgG (0.18 nM) was incubated as described in Fig. 1 in the presence of various amounts of human erythrocytes (●), neutrophils (○) and platelets (▲) (a), and, in a separate experiment, with human erythrocytes (●), cultured foreskin (■) and Wi-38 lung (○) fibroblasts. Binding of ^{125}I -labelled 72,000-MW fragment (3.6×10^6 c.p.m. pmol^{-1} , 0.6 nM) was then determined (Fig. 1). Peripheral blood cells were separated into erythrocytes, neutrophils and a combined lymphocyte and platelet fraction by the method of Boyum¹⁰. Lymphocytes and platelets were separated by sedimentation on sucrose gradients¹¹. Cultured human fibroblasts from a foreskin explant and from embryonic lung (Wi-38) were grown to confluency in Eagle's minimum essential medium supplemented with 10% (v/v) fetal calf serum, and were detached after exposure to 150 mM NaCl and 5 mM Na EDTA, pH 7.5. The erythrocytes in these assays contain the equivalent of 100,000 ankyrin molecules per cell based on a radioimmunoassay with SDA-denatured cells and comparison with a standard curve of 72,000-MW fragment.

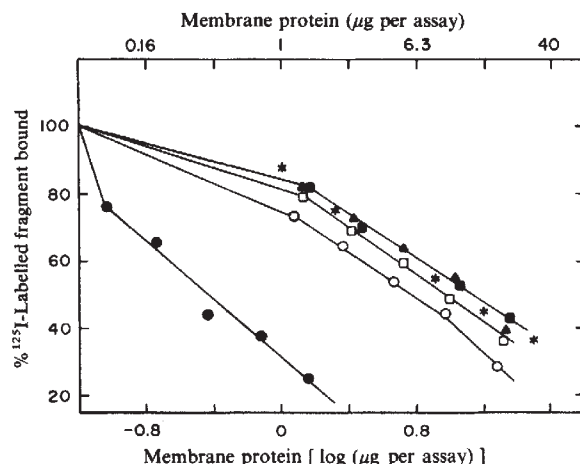


Fig. 3 Inhibition of binding of ^{125}I -labelled 72,000-MW fragment to monospecific anti-fragment IgG by membrane protein from rat erythrocyte ghosts, brain, liver, kidney, testes and epididymal fat cells. Anti-fragment IgG (0.23 nM) was incubated in the presence of various concentrations of membrane protein from rat erythrocyte ghosts (●), brain (○), liver (■), kidney (▲), testes (□) and epididymal fat cells (*), and binding of ^{125}I -labelled fragment (2×10^6 c.p.m. pmol^{-1} , 0.5 nM) was then determined (Fig. 1). Membrane fractions from rat tissues were prepared by homogenising 1 g of perfused liver or kidney, brain cerebrum, testes or isolated fat cells from eight 100-g rats¹² in 9 ml of 0.25 M sucrose, 2 mM Na EGTA, pH 7.0, and 200 $\mu\text{g ml}^{-1}$ phenylmethylsulphonyl fluoride with a Brinkman polytron (30 s, 3,200 r.p.m.); the suspensions were pelleted for 10 min at 900g to remove nuclei, large particles and possible contaminating erythrocytes. The supernatants were then diluted 10-fold and centrifuged for 30 min at 250,000g to collect the membrane fractions.

erythrocyte ankyrin is released from the membrane with very limited proteolysis⁵.

The possibility of contamination of the membrane fractions from solid tissues by erythrocytes was minimised by using conditions for homogenising the tissues that lyse only 10–15% of erythrocytes, and including a low-speed centrifugation step to remove unbroken cells before isolation of the membranes. The amount of contamination by intact and lysed erythrocytes in these low-speed supernatants was estimated by solubilising membranes with Triton X-100, and measuring haemoglobin by absorbance at 578 nm. The values obtained by this assay corresponded to a maximum contamination of membranes with erythrocyte ankyrin of 0.005% for brain, 0.009% for liver, 0.008% for kidney and 0.006% for testes. The activity of these membrane fractions thus cannot be explained by contamination with erythrocyte membranes. Furthermore, fat cells have a density of less than 1 g cm^{-3} , and consequently float whereas other cells are sedimented. These buoyant cells are thus completely free of erythrocyte contamination, and still contain the same amount of immunoreactivity as liver and kidney.

The small amounts of immunoreactivity in these membrane fractions, and the extreme sensitivity of ankyrin to proteolysis, makes a detailed analysis of the cross-reacting polypeptides quite difficult. However, it was possible to immunoprecipitate with anti-ankyrin IgG a polypeptide of MW $\sim 200,000$ from ^{125}I -labelled brain and fat cell membranes (not shown). This polypeptide was not precipitated in the presence of excess, 72,000-MW ankyrin fragment, nor by preimmune IgG. Many other polypeptides were also present in the immunoprecipitates, but their precipitation was not reduced by the ankyrin fragment, and they were also obtained with preimmune IgG.

The molecular weight of erythrocyte ankyrin is similar to that of myosin and of filamin. These proteins, however, did not contribute to the cross-reactivity in these various cell types, as partially purified myosin or filamin from human platelets were not active in the radioimmunoassay. Furthermore, the total

protein fraction from rat skeletal myofibrils exhibited very low activity.

These results demonstrate the ubiquitous presence of small amounts of an ankyrin-like protein distinct from myosin or filamin which is associated primarily with membrane components. Ankyrin links band 3 to the erythrocyte cytoskeleton⁴, and may have a related function in specialised regions of the plasma membrane in other cells. Spectrin was not detected in cultured cells by radioimmunoassay¹⁴, but this negative result could be explained by poor cross-reactivity of the antibody or by insufficient sensitivity of the assay. It is conceivable that proteins analogous to ankyrin, spectrin and band 3 could be present together in non-erythrocyte cells, and could be involved in processes requiring direct contact between integral membrane proteins and structural proteins on the inner surface of the membrane.

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A mutation in *Caenorhabditis elegans* that increases recombination frequency more than threefold

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In higher organisms the rate of recombination between genetic loci is presumably responsive to selective pressure. Recently, selective pressures¹ and mutational events² that influence recombination have been reviewed. Mutational sites and chromosomal rearrangements that enhance or suppress recombination frequency in specific regions are known, but general mechanisms that enhance recombination have not yet been discovered. We describe here the isolation and characterisation of a strain of the hermaphroditic nematode, *Caenorhabditis elegans*, that has a recombination frequency at least threefold higher than that found in the wild type^{3,4}. In this strain, *rec-1*, the number of reciprocal recombination events between linked loci is increased. This is true for all pairs of linked loci studied so far. The high recombination strain behaves as if it carries a classical, recessive mutation, although a second mutation exists which can alter the recessive behaviour of *rec-1*.

We established a strain heterozygous for mutations in closely linked loci on chromosome I. This strain, *dpy-5 unc-13* *ab* *+/++ unc-13*, has a wild-type appearance. When allowed to self-cross it segregates dumpy paralysed (*dpy-5 unc-15/dpy-5 unc-15*), uncoordinated (*unc-13/unc-13*) and wild-type appearing (*dpy-5 unc-15* *+/++ unc-13*) progeny. *dpy-5 unc-15/dpy-5 unc-15* are short, fat worms that cannot move, and *unc-13/unc-13* have a characteristic contorted appearance and movement. In addition to these phenotypes, recombinant dumpy (*dpy-5* *+/dpy-5 unc-15*) and paralysed (*+/unc-15/dpy-5 unc-15*) progeny will occur. By selecting the hermaphrodites with wild-type appearance, this strain was maintained for

Table 1 The effect of *rec-1* on recombination frequency (%)

<i>ab</i>	Parental genotypes		
	<i>ab</i> <i>+/+</i>	<i>ab</i> <i>+/+</i> ; <i>rec-1</i> <i>+/+</i>	<i>ab</i> <i>+/+</i> ; <i>rec-1</i> <i>rec-1</i>
<i>dpy-5 unc-13</i> (I)	1.51 ± 0.3*	1.30 ± 0.3	12.41 ± 1.6
<i>dpy-14 unc-56</i> (I)	†	0.61 ± 0.2	4.14 ± 1.5
<i>unc-22 unc-43</i> (IV)	1.90 ± 0.6	1.55 ± 0.4	3.95 ± 1.4
<i>dpy-11 unc-42</i> (V)	2.21 ± 0.3	2.60 ± 0.7	7.92 ± 1.8

* ± 95% confidence intervals.

† Not determined.

several generations, and eventually scored for recombination between the *dpy-5* and *unc-15* loci. The frequency of recombination in this region was known from previous experiments⁴ to be approximately 2% at 20 °C when all the progeny of a single parent were scored. In the heterozygous strain that we were maintaining, the recombination frequency between *dpy-5* and *unc-15* was approximately 7%. We hypothesised that our *unc-13/unc-13* strain, used to make the heterozygous strain, carried a factor responsible for this increase in recombination frequency. To demonstrate the existence of this factor, we out-crossed our uncoordinated strain to wild type and generated *unc-13* *+/+* males. These males were crossed to *dpy-5 unc-15/dpy-5 unc-15*. On self-crossing, half the F₁ heterozygotes from this cross showed a high frequency of recombination among their progeny, while the other half had a normal recombination frequency. Successive generations of the high recombination individuals were measured for recombination frequency until a homozygous strain of high recombination hermaphrodites (XX) was established. This strain was treated at 26 °C for 30 h to produce males (XO) by X-chromosome nondisjunction. When these males were crossed to *dpy-5 unc-15/dpy-5 unc-15*, all the F₁ heterozygotes segregated a high number of recombinant individuals. After elimination of *dpy-5*, *unc-15* and *unc-13* mutant alleles by recombination, we established a male strain homozygous for *rec-1*, but having no visible mutant phenotype.

Table 2 The influence of *s155* on the expression of *rec-1*

<i>ab</i>	Parental genotypes	
	<i>ab</i> <i>+/+</i> ; <i>s155</i> <i>+/+</i>	<i>ab</i> <i>+/+</i> ; <i>s155</i> <i>+/+</i> ; <i>rec-1</i> <i>+/+</i>
	Recombination (%)	
<i>dpy-5 unc-13</i> (I)	1.15 ± 0.3	7.34 ± 0.9
<i>dpy-5 unc-15</i> (I)	1.80 ± 0.4	9.11 ± 0.9
<i>dpy-5 dpy-14</i> (I)	1.62 ± 0.6	5.50 ± 0.9

Males from this strain were crossed to *dpy-5 unc-13/dpy-5 unc-13* individuals. Unlike before, all the F₁ heterozygotes produced a normal number of recombinants among their progeny. In the F₂, two types of heterozygote occurred—those which gave a normal frequency of recombinants and those which gave a high frequency of recombinants. Approximately one-quarter of the F₂ heterozygotes had high recombination frequencies and produced true-breeding high recombination progeny. In a parallel control experiment, *dpy-5 unc-13/dpy-5 unc-13* were crossed to *+/+* males and scored for recombination frequency in the F₁, F₂ and F₃. In this case the recombination frequency remained approximately 2% in every generation. From these studies, we concluded that the *rec-1* strain has a segregating factor which assort independently of chromosome I. This factor is responsible for at least a threefold elevation in recombination frequency.

To investigate the generality of this increased recombination phenomenon, *rec-1/rec-1* males were crossed to other pairs of linked loci on chromosomes I, IV and V. The results showed that *rec-1* acts recessively to increase recombination frequency between two pairs of linked loci on chromosome I (Table 1). On

chromosomes IV and V, recombination frequency in the *unc-22 unc-43* and the *dpy-11 unc-42* regions, respectively, was measured. Here also, an increase in recombination frequency was observed (Table 1). We interpret these results as evidence that *rec-1* is a general enhancer of recombination frequency in *C. elegans*.

With certain pairs of linked loci, a high recombination frequency was observed in the F_1 generation. For example, when *rec-1* males were crossed to *dpy-5 unc-15/dpy-5 unc-15* or *dpy-5 dpy-14/dpy-5 dpy-14*, the F_1 heterozygotes had a higher recombination frequency than wild type. From these results we hypothesised that *dpy-5 unc-15/++* and *dpy-5 dpy-14/++* individuals carry a site, *s155*, responsible for the dominant behaviour of the high recombination factor.

A strain, homozygous for *dpy-5 unc-13* and *s155* was constructed. If *s155* is, in fact, responsible for conferring dominant behaviour on *rec-1*, then *s155*-carrying *dpy-5 unc-13/dpy-5 unc-13* individuals should behave differently from *dpy-5 unc-13/dpy-5 unc-13* individuals when crossed to *rec-1*. Table 2 shows that when pairs of loci on chromosome I are tested in the presence of *s155*, *rec-1* acts dominantly. This provides evidence that a second mutation can modify the recessive nature of *rec-1*. Experiments are in progress to test whether *s155* can confer dominance on regions on other chromosomes.

We believe this to be the first demonstration of a general enhancer of recombination frequency in a higher eukaryote. The existence of a gene that can alter recombination and that is susceptible to mutation provides a mechanism whereby recombination frequency can be selected. It may be possible that, in addition to this allele of *rec-1*, other alleles which decrease recombination frequency can be recovered. If so, perhaps different levels of recombination frequency are selected in exclusively self-crossing or exclusively out-crossing populations. Furthermore, our *rec-1* strain may have an elevated spontaneous mutation rate. Three new visible phenotypes independent of *rec-1* have been observed in this strain. Thus, it is possible that the mechanism by which *rec-1* acts also alters the mutation frequency. This and other questions regarding the mode of action of *rec-1* are being investigated.

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Correlation between fragmented immunoglobulin genes and heavy chain deletion mutants

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It is generally accepted that the variable (V) and constant (C) regions of immunoglobulin (Ig) chains are under separate genetic control¹. The notion that the different domains and interdomain regions are also under the control of independent genetic units was initially based on the clearcut results obtained by studying the primary structure of deletion mutants²⁻⁹ and received definitive support from direct analysis of cloned heavy (H) and light (L) chain genes¹⁰⁻¹³. Here we present additional studies carried out on two selected $\gamma 3$ deletion mutants which indicate that the genetic control of human H chains may be even more complex than previously believed.

L and H chains of Ig molecules consist of two functionally differentiated segments: the amino-terminal V region which contains the antigen binding sites, and the carboxy-terminal C region¹⁴. Sequence and crystallographic studies have shown that the C region of the IgG H chain is composed of three domains: CH1, CH2 and CH3. The striking structural homology among the C region domains, each of which is composed of about 110 residues folded into an almost identical tertiary structure, and the lesser homology to the V regions suggest a common evolutionary origin by a series of gene duplications. Each domain has a well defined function. The CH1 domain contains the attachment point for the L chain and pairs with the CL domain; together with paired VH and VL domains containing the antigen binding sites they form the Fab fragments of the IgG molecules. The CH2 domains, which act in complement fixation, and the CH3 domains, which are involved in cell surface interactions together form the Fc fragment. In the middle of the H chains of all subclasses of IgG, there is a region of 15-62 residues of unknown origin and function called the hinge region or Fh fragment, which separates the Fab and Fc fragments^{15,16}. Thus, the γ H chain is composed of five discrete structural and functional units.

Heavy chain disease (HCD) protein SPA is an 80,000-molecular weight (MW) dimeric $\gamma 3$ human H chain mutant with a blocked amino terminus. The amino-terminal sequence after unblocking is: <Glu-Glu-Glu-Val-Arg-Glu-Ser-Glu-Leu-Lys-Thr-Pro-Leu-Gly-Asp-Thr-Thr-(His)-Thr-Cys-Pro-(Pro)-Cys-Pro. Comparison with the prototype VH amino-terminal sequences shows that although the first seven residues bear some resemblance to the normal amino terminus they do not correspond to any known VH subgroup or CH1 sequence²⁵. Position 8 is the beginning of the normal $\gamma 3$ hinge region¹⁶. On the basis of its amino acid analysis and high cysteine content it seems

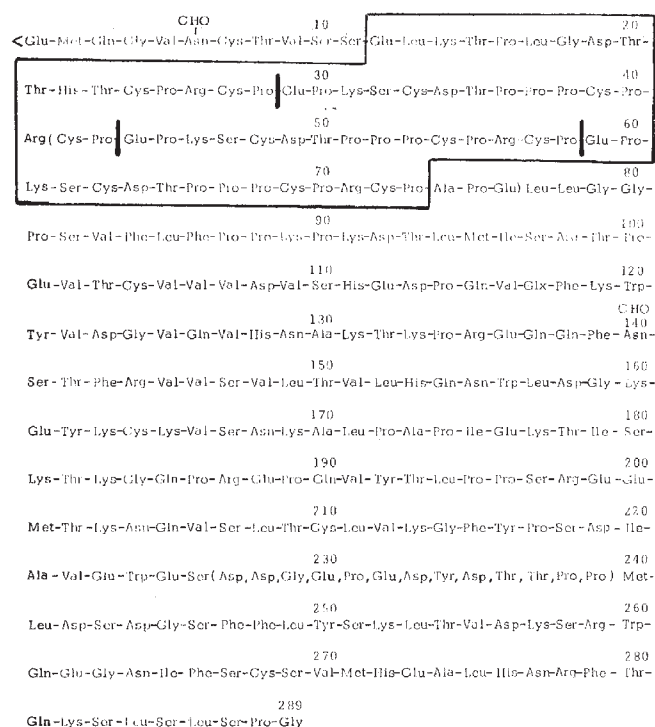
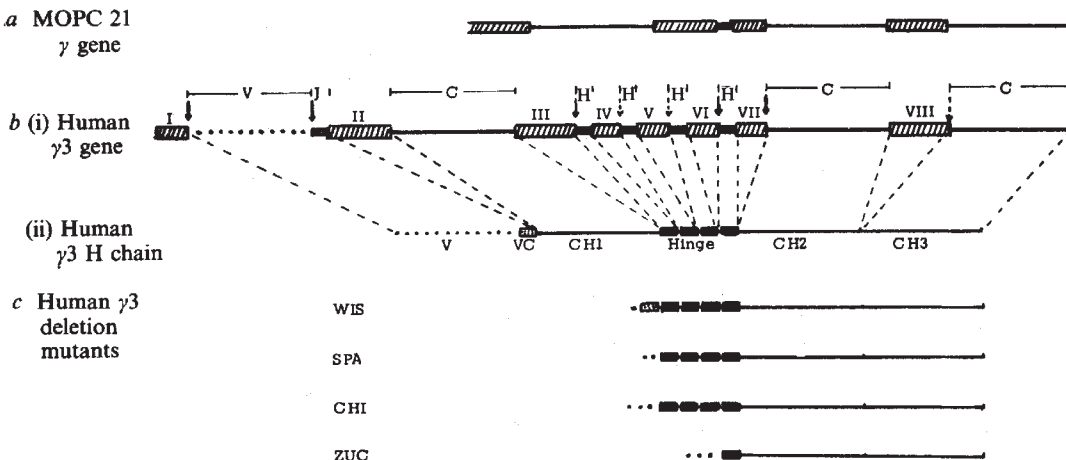


Fig. 1 The amino acid sequence of human $\gamma 3$ heavy chain deletion mutant WIS. Position 1 to 7 is an unusual V sequence; position 8 to 11 corresponds to VC or junction sequence; position 12 is the beginning of the hinge region of normal $\gamma 3$ heavy chain which is 62 amino acid residues long (inside box) and four homologous subunits indicated by vertical bars. The first is a 17-residue segment followed by a 15-residue segment which is identically and consecutively repeated three times. CHO: carboxyhydryte; <Glu: 5-pyrrolidone-2-carboxylic acid.

Fig. 2 Organisation of immunoglobulin H chain genes. Correlation with $\gamma 3$ chain and $\gamma 3$ deletion mutants. *a*, Arrangement of genes coding for MOPC 21 $\gamma 1$ H chain constant region¹³. *b*(i), Proposed arrangement for human $\gamma 3$ genes: ... coding DNA for V region; —, coding DNA for C domains; ■, coding DNA for J junction of VC region; ▨, coding DNA for hinge or H junction region; ▩, (IVS) intervening sequences. IVS II, III, VII and VIII are present in the cloned DNA fragment coding for the murine $\gamma 1$ constant region¹³, and IVS I–VII are suggested by the structure of $\gamma 3$ deletion mutants and myeloma proteins with a deleted hinge^{5,23,24}. ↓, Indicate splicing sites as defined by deletion mutants; ↓, not observed in man. *b*(ii), Human $\gamma 3$ heavy chain. ... V domain; ▩, VC region; ▨, hinge region (quadruplicated); —, CH1, CH2, CH3: constant domains. *c*, $\gamma 3$ deletion mutants. Heavy chain disease proteins WIS, SPA, CHI⁷ and ZUC^{2,17}. For a complete list of deleted H chains see refs 6 and 9 for mouse and human, respectively.



likely that the intact hinge and the entire Fc fragment are present and that protein SPA has an internal deletion of about 210 residues comprising most of the V, the VC or J (junction) and CH1 regions.

HCD protein WIS is an 86,000-MW protein composed of two identical 289-residue chains. Figure 1 shows its amino acid sequence (details will be published elsewhere). It has two carbohydrate moieties attached to Asn 6 and Asn 140 and position 7 contains an unusual cysteine residue. Homology of the first 11 residues to both VH and the VC junction region has been previously pointed out⁸. The size of the deletion is about the same as that of protein SPA as position 12 marks the beginning of a normal hinge, consisting of 62 residues composed of 4 homologous regions. The hinge starts with a 17-residue segment (residues 12–28) followed by a 15-residue segment which is identically and consecutively repeated three times (residues 29–43, 44–58 and 59–73). The sequence from residue 74 to the carboxy terminus seems to be similar to that of previously reported Fc fragments¹⁷.

The unexpectedly high frequency of deletion mutants belonging to the $\gamma 3$ subclass allows conclusions of general significance. Figure 2c compares protein WIS and SPA with two other previously reported $\gamma 3$ deletion mutants, protein CH1 (ref. 7) and ZUC (refs 2, 17). The most constant feature of γ -chain deletion mutants is the presence of an internal deletion involving part of the VH and usually all of the CH1 domains which usually ends at the hinge. In the case of $\gamma 3$ mutants, the deletion can end either at the beginning of quadruplicated hinge, or in one case (ZUC), at the beginning of one of the segments. In contrast, in different γ heavy chain deletion mutants the amino terminus can be normal—similar but not identical to—the known VH subclasses, as is the case in the proteins reported here, or very different from the usual amino terminus³. This complexity precludes any generalisations as to possible mechanisms responsible for these findings and has been discussed previously¹⁸. The constancy of the remainder of the Fc fragment as reflected by the relatively infrequent occurrence of additional mutations in CH2 and CH3 domains has also been emphasised before and is further supported by the sequence of protein WIS.

The unique structure of the hinge contrasts with the striking homologies of the four H chain domains. Its basic subunit is 15 residues long, rich in proline and contains the inter-heavy chain disulphide bridges and sometimes the HL bridge¹⁵. Its origin is unknown; it could be part of a gene coding for a domain which has undergone deletions and mutations, it could be encoded by intervening sequences shown to be present between genes coding for domains¹³, or as previously suggested it may be an

inserted DNA fragment (transposon) capable of joining duplicated genes which have evolved to perform different biological functions⁴, somewhat analogous to the J fragment of the L chain¹¹. These features, together with the finding that the hinge often marks the end of the internal deletions⁹, that it can be deleted selectively^{5,9}, and that it is a site where duplications and unequal crossing over occur¹⁶, have given rise to the suggestion that the hinge may be under the control of a separate genetic unit. This possibility has received strong support from the recent observation that non-coding DNA segments occur not only between the domains but also at each side of the hinge in the case of cloned DNA for murine γ -chains¹³ (Fig. 2a).

Eukaryotic genes, including immunoglobulin L and H chain genes, are organised into transcriptional units in which coding segments of DNA (exons) alternate with non-coding sequences (intervening sequences or introns) to be excised from the primary transcript^{10–13,19}. The organisation of the immunoglobulin genes seems to be unique in that the intervening sequences apparently separate exons coding for structural subunits or domains. In the case of the cloned DNA coding for the constant region of the mouse $\gamma 1$ heavy chain, electron microscopy and sequence analysis revealed four exons separated by four intervening sequences¹³ (Fig. 2a). Comparison with the known amino acid sequence²⁰ demonstrated that the exons represent the three CH domains and the hinge, and that no other intervening sequences exist within coding DNA segments. However, because of the complexity of human $\gamma 3$ hinge gene(s), additional intervening sequences and splicing sites can be anticipated between the subunits of its quadruplicated hinge region (Fig. 2b, H region). In addition, based on the unusual V region sequences in γ deletion mutants and the sequence of protein WIS (see Fig. 1, residues 8–11), intervening sequences similar to those described for the mouse λ -chain gene^{10,11} (Fig. 2b) may also exist near the amino terminus of the V gene and at the J region. Because the intervening sequences are excised from the precursor and the coding regions rejoined, there must be some recognition signal at the junction between the intervening and the coding sequences to permit the accurate excision and splicing necessary to produce the mature mRNA²¹. Such seems to be the case for the repetitive subunits of the hinge genes which, as pointed out before, have kept sequence signals similar to those at the joining sites between VJ and C genes of the L chains²².

In both H and L chain deletion mutants there is a striking correlation between the borders of deletions and the recently defined points of splicing. Figure 2 correlates the splicing points defined for mouse $\gamma 1$ constant genes with sites previously described for recombination in deleted H chains. There is also similar striking correspondence for fragmented L chain genes

and two L chain mutants⁸ which end at the intervening sequences bordering the J region. If sites of splicing determine where deletions can end, as seems to be the case in all mutants studied so far, the finding that in protein ZUC the deletion ends at the last subunit rather than the beginning of the quadruplicated hinge provides additional support for the existence of splicing points and intervening sequences between the subunits of the hinge (Fig. 2b).

Although the mechanisms responsible for the deletions are not known, the defect could occur either at the DNA level or during splicing events when heterogeneous nuclear RNA is converted to mature mRNA. A mutation at or near a splicing site could alter the splicing pattern so that the entire coding region can be skipped in the mRNA; alternatively, if the splicing enzyme(s) can bridge non-adjacent encoded regions, large deletions involving more than one genetic unit could result in mutant proteins in which more than a single domain can be eliminated. Studies of immunoglobulin variants, together with DNA cloning of selected mutant cells, will undoubtedly continue to provide clues to the genetic mechanisms involved in H and L chain control and pose interesting questions about the evolution and significance of eukaryotic fragmented genes.

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Suppression of the nonsense mutation in homozygous β^0 thalassaemia

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The common form of β thalassaemia associated with elevated haemoglobin A₂ levels can be broadly classified as β^+ or β^0 type according to the presence or absence of β -globin chain synthesis in the homozygous state^{1–4}. The molecular pathology of each type is heterogeneous^{3–9}. Apart from a subgroup of Indo-Pakistani patients¹⁰, the β -globin structural gene is intact in the majority of patients with β^0 thalassaemia^{2–4}. The amount of

β -globin mRNA present in the reticulocytes of these patients varies^{5–7}: in some it is absent or barely detectable; in others, a substantial amount is present, but it is nonfunctional. We recently demonstrated that the molecular lesion in a Chinese patient with nonfunctional β -globin mRNA^{11,12} was due to the mutation of the normal lysine codon AAG at amino acid 17 to the amber terminator codon UAG, which prematurely terminates the β -globin chain¹³. In the present study we demonstrate the first example of a nonsense mutation in humans which can be suppressed *in vitro* by the suppressor tRNA, as has been found in other eukaryotic cells and viruses^{14,15}.

The *in vitro* translation of the globin mRNAs prepared from the reticulocytes of the patient with homozygous β^0 thalassaemia was performed in the micrococcal nuclease-treated rabbit reticulocyte lysate system using ³⁵S-methionine as the radioactive amino acid¹⁶. The globin chains synthesised were separated on a carboxymethyl cellulose column¹⁷ (Fig. 1). The patient's mRNAs directed the synthesis of α - and γ -globin chains but no β -globin chain in the rabbit reticulocyte system. When a serine-inserting amber suppressor tRNA which recognises the codon UAG was added to the mRNA in the cell-free system a new radioactive peak appeared. Its radioactivity was about one-twentieth of that of α -globin. This new polypeptide chain eluted from the column earlier than the β^A -globin marker, consistent with the insertion of serine instead of lysine in amino acid number 17 by the serine suppressor tRNA. We did not detect any additional peaks as the normal termination codons of α -(UAA) (ref. 18) and γ -globin (UGA) (ref. 19) would not be suppressed by this tRNA. As a control, we added this suppressor tRNA to the mRNA from a patient with sickle cell anaemia and detected no new radioactive peak (data not shown).

To confirm that the newly synthesised polypeptide from the β^0 thalassaemia mRNA was a β -globin chain, we digested the polypeptide with trypsin, separated the tryptic peptides by high voltage paper electrophoresis²⁰, and identified the ³⁵S-methionine-containing peptide, β T5 (amino acid numbers 41–59). The β T5, from the newly synthesised chain, migrated to the same position as that of the β^S chain, confirming that it was indeed a β -globin chain (Fig. 2).

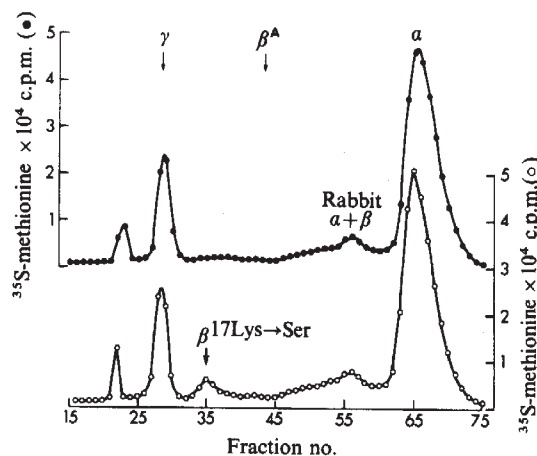


Fig. 1 Chromatograms of ³⁵S-methionine-labelled globin chains. The patient's mRNA was assayed in the micrococcal nuclease-treated rabbit reticulocyte lysate system using the method of Pelham and Jackson¹⁶. Each 50 μ l reaction contained 47 μ l of the treated lysate, 100 nmol ³⁵S-methionine (Amersham, 1,000 Ci mmol⁻¹), 1 μ g of the mRNA purified by two passages on oligo (dT)-cellulose columns¹², without (●) and with (○) the addition of 6 μ g of serine-inserting, amber suppressor tRNA from *Saccharomyces cerevisiae*. The suppressor tRNA was isolated from the amber suppressing strain DB339 and purified by BD-cellulose and Sepharose 4B column chromatography²³. The globin chain products were separated on a carboxymethylcellulose column as previously described¹⁷. The position of elution of normal γ -, β^A - and α -globin chains was determined by optical density measurements of 5 mg of HbA and F added as carrier.

The amount of β -globin mRNA present in this patient's reticulocytes was 15% of the α -globin mRNA⁴. Since the efficiency of the suppressor is generally not more than 60% (refs 21, 22), the amount of β -relative to α -globin synthesis would be about 10%. The finding of a β : α ³⁵S-methionine radioactivity ratio of 1:20 is consistent with this since β -globin chain contains one, and the α -globin two methionine residues. The low level of β -globin mRNA may be due to premature degradation of the mRNA, as a result of lack of ribosomes over nearly 90% of the coding region following the nonsense mutation. Such a mRNA would be more susceptible than normal to nuclease degradation within the erythroid cell. In addition, the decreased β -globin mRNA could also be partially explained if one of this patient's β -globin alleles contained the nonsense mutation while the other contained a different kind of mutation which results in the absence of mature β -globin mRNA from that gene.

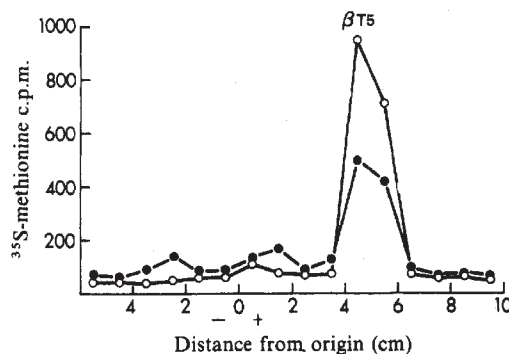


Fig. 2 High voltage electrophoresis of ³⁵S-methionine-labelled tryptic peptides. The β -globin peaks from cell-free synthesis products with mRNA from the β^0 thalassemic patient (●) and a patient with sickle cell anaemia (○) were desalted through a Sephadex G-25 column in 0.5% formic acid, and lyophilised. 200 μ g of globin from each peak was incubated in 0.5 ml of 1% NH_4HCO_3 at 37°C with 8 μ g of trypsin (Worthington TPCK-treated, 259 units mg^{-1} for 4 h. The hydrolysate was twice lyophilised and dissolved in 10 μ l of pyridine acetate buffer (pyridine: acetic acid: water 25:0.3:75 by volume), pH 6.58. The peptides were separated by electrophoresis on 3 MM paper at 2,000 V for 2 h, 1-cm strips of the paper were cut out and the radioactivity measured in a Beckman liquid scintillation counter.

This study corroborates our sequence analysis data on this patient which indicated that β^0 thalassaemia is the result of a nonsense mutation within the β -globin structural gene¹³. To date, only one likely example of a nonsense mutation in higher eukaryotic cells has been reported. Capecchi *et al.* described a mouse cell line deficient in the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT)¹⁴. The mutant enzyme was probably shortened at the carboxy terminus. Fusion of the defective cells with erythrocytes preloaded with either *Escherichia coli* or yeast suppressor tRNAs restored HGPRT activity when the erythrocytes contained ochre (UAA) but not amber (UAG) suppressor tRNA. Our results indicate that a defined nonsense mutation in human β -globin mRNA can be suppressed *in vitro* using the appropriate yeast suppressor tRNA. Similarly, delivery of suppressor tRNA to the erythroid cell precursor of patients with β^0 thalassaemia due to nonsense mutations may correct the β -globin defect. This approach may offer new methods for treatment of this disorder.

It is likely that nonsense mutations may have occurred at different locations along the β -globin mRNA in other β^0 thalassaemias. The use of suppressor tRNAs will facilitate the detection of nonsense mutations in the various thalassaemia syndromes as well as in other genetic disorders where a specific protein is deficient or absent.

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Heterozygosity of *H-2* loci in wild mice

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The major histocompatibility complex (MHC) is a cluster of genetic loci coding for cell surface molecules that seem to be the second most important element of the vertebrate immune system after immunoglobulins¹. The MHC loci can be divided into three classes. Class I loci code for molecules of about 44,000 molecular weight (MW) which seem to function as markers of self in T-lymphocyte-mediated cytotoxicity. Class II loci code for molecules consisting of two polypeptide chains, of MW 34,000 and 28,000 respectively, which are believed to regulate the immune response by controlling the behaviour of helper and suppressor T lymphocytes. Each molecule may carry several antigens—one 'private' (characteristic for the allele controlling this molecule) and several 'public' ones² (shared with molecules controlled by other alleles). Class III loci code for serum proteins. One of the most characteristic and remarkable properties of the MHC loci, in particular class I, is their genetic polymorphism, defined as the existence in a population of two or more alleles at appreciable frequencies³⁻⁵. In *H-2*, the MHC of the mouse, individual alleles at class I *K* and *D* loci occur in the two populations that have been studied thus far with an average frequency of about 2% (ref. 5). We estimate, therefore, that these two populations contain some 100 alleles at each of the two loci. The polymorphism of class II locus *A* seems to be somewhat lower than that of the class I loci (average allelic frequency of 2-5%), while at the second class II locus (*E*) only five alleles have been identified⁵. The polymorphism implies that a high degree of heterozygosity should exist at the MHC loci in natural populations, and this has been found at the *HLA* loci, the human MHC (for review see ref. 6). We report here a similarly high frequency of heterozygosity in the mouse *H-2* complex in a population of mice, in spite of a social structure that would tend to reduce heterozygosity.

Table 1 Class I H-2 antigens of wild mice and their progeny

Wild mouse	No. of progeny	Phenotype	Wild mouse	No. of progeny	Phenotype
CC27	—	105, 115, 117	HGE61	—	2
	2	105, 115, 117		1	2
	3	—		3	—
DF55	—	17, 103, 118	AU136	—	19
	1	17		2	19
	1	103, 118		4	—
WD104	—	9, 103, 118	KF72	—	2
	2	103, 118		1	2
	1	9		1	—
DFW36	—	9	DF85	—	20, 105, 114
	3	9		2	20
	2	—			
DFW33	—	9, 20, 103, 116, 118	OS9	—	16, 115, 117
	2	9, 20, 103, 118		1	16
	2	116		2	115, 117
PD121	—	119, 120	CC16	—	118, 119, 120
	2	119, 120		1	118, 119, 120
	3	—		3	—
DF48	—	119, 120	MD3	—	108, 111, 119, 120
	1	119, 120		3	108, 111, 119, 120
	3	—		1	—
DF89	—	116	DF45	—	25, 118
	2	116		1	118
	2	—		1	25, 118
CC21	—	115, 117	DF92	—	2, 16, 117, 119, 120
	2	115, 117		3	119, 120
	2	—		2	2, 16, 117
MS8	—	105, 115, 117	BCD131	—	119, 120
	1	105		1	119, 120
	1	115, 117		3	—
MD7	—	19	DWD130	—	103, 118
	1	19		3	103, 118
	1	—		1	—
OS40	—	16, 31, 105, 115, 117	DF88	—	20, 25, 109
	3	16		6	20, 25, 109
	1	31, 105, 115, 117			

Wild males were mated with MWT (*H-2^b*) females and tested, together with their progeny, in the cytotoxic test against a panel of operationally monospecific antisera. Positive reactions for private and semiprivate antigens listed under Phenotype were found.

There are two ways of estimating *H-2* heterozygosity. First, if all private *H-2* antigens and the loci coding for them in a given wild mouse sample were known, one could determine the heterozygosity of each locus simply by counting individuals carrying two allelically determined private antigens. Because, however, we do not know all the private *H-2* antigens and we often do not know the identity of loci (*H-2K* versus *H-2D*) controlling them, we can only use this method to estimate the minimal frequency of *H-2* heterozygotes. As there are two major class I and two major class II loci, the presence of three private class I or class II antigens in an individual must mean that this individual is heterozygous in at least one class I (or class II) locus. Similarly, simultaneous presence of four private class I or class II antigens in an individual can be interpreted to mean that this individual is heterozygous at the two class I or class II loci. Individuals with two or less private antigens may or may not be heterozygotes, and it is this uncertainty that makes the estimate minimal.

Wild mice were captured at 15 different localities in Texas, and typed in the dye-exclusion cytotoxic test with 49 *H-2* antisera and 12 Ia sera, which detected 36 private class I and 10 class II antigens⁵. In this sample of 88 mice, 31 mice carried more than two private class I antigens (data not shown). Thus, the minimal frequency of *H-2* heterozygotes at class I loci among Texas mice is 35%.

The second way of estimating *H-2* heterozygosity involves crossing wild males (wild female mice do not usually mate in captivity) with an *H-2* homozygous testing stock of laboratory mice, and *H-2*-typing the progeny of this cross. Segregation of

private *H-2* antigens among the progeny indicates heterozygosity of the wild father. This method fails only in cases where the father does not carry any known private antigens or where one of the paternal *H-2* chromosomes carries a strong segregation distorter, such as that present in the *H-2* linked *t* complex⁷.

This second approach was applied to 26 of the mice captured in Texas, which were mated with *H-2^b* homozygous MWT stock. From these matings 90 hybrids and their fathers were obtained and typed with the above panel of *H-2* and Ia antisera (Table 1). Two of the 26 wild males (and their progeny) did not carry any of the private class I antigens detected with our battery of antisera. Of the remaining 24 males, private *H-2* antigens segregated in the progeny of 23; the offspring (six) of only one mouse (DF 88) all carried the same class I antigens as their father. Provided that this result was not caused by segregation distortion, this one mouse might have been an *H-2* homozygote. Thus, the overall frequency of class I heterozygotes in the sample was 96%. In seven of the wild fathers private antigens were present that are known to be controlled by the *H-2K* locus. All seven mice proved to be heterozygous at this locus. Similarly, in four mice, antigens known to be controlled by the *H-2D* locus were found, and these mice were also all *H-2D* heterozygotes. With regard to class II antigens, it was possible to type 10 mice for Ia-1 and 19 mice for Ia-5 antigens. Eight of the Ia-1 and nine of the Ia-5 mice were heterozygous. Hence, the frequency of heterozygotes at Ia-1 and Ia-5 loci is 80 and 47%, respectively (Table 2).

This high degree of heterozygosity is unprecedented in a wild mouse population. The average frequency of heterozygotes at all other polymorphic loci tested is about 6%, with the highest recorded frequency of 28.6% for malic enzyme⁸. The *H-2* far exceeds this frequency. In fact, in the *H-2* system, maximum heterozygosity is achieved in that, at least for the class I loci, virtually all the mice are heterozygous.

Table 2 Number of wild mice heterozygous at individual *H-2* loci

Locus	Heterozygous total tested	% Heterozygotes
<i>H-2K</i>	7/7	100
<i>H-2D</i>	4/4	100
Class I*	23/24	96
Ia-1	8/10	80
Ia-5	9/19	47
All <i>H-2</i> †	25/26	96

*Includes mice in which *H-2K* and *H-2D* loci could not be distinguished phenotypically.

†If one of the four loci tested was heterozygous, the mouse was counted as a heterozygote.

How does this high *H-2* heterozygosity of natural mouse populations arise? Were the mouse population of the random mating type, such a high frequency of *H-2* heterozygotes would be expected. If the frequency of individual *H-2* alleles is—as preliminary data⁵ suggest—about 2%, and if there are approximately 100 alleles at each of the three highly polymorphic *H-2* loci (*H-2K*, *H-2D* and Ia-1), then the expected frequency of *H-2* heterozygotes would be nearly 100%. However, it is known that mouse populations are not of the random mating type. On the contrary, they are broken down into family units or demes, each family consisting of one dominant male, a few subordinate males and a few females (for review see ref. 9). The demes are more or less closed, with little evidence for interdemec migration. Although there is almost no evidence indicating that mice in Texas live in deme-type populations, there is no reason to believe that they are different in this respect from mice in other geographical areas. Furthermore, preliminary data on mice from Germany indicate that these too display a high degree of *H-2* heterozygosity (unpublished). Thus, the phenomenon seems to be of general validity.

The deme structure of the mouse population and the high degree of heterozygosity need to be reconciled. There is considerable evidence indicating that mice within a deme (or locality) genetically resemble one another more than mice of different demes (see ref. 9). This observation, which also applies to minor histocompatibility loci¹⁰ (that is, *H* loci that are not members of the MHC), suggests that a certain degree of inbreeding occurs within a deme. The intrademe inbreeding should lower the heterozygosity at the *H-2* loci, the degree of the decrease depending on the inbreeding coefficient. As the latter is not known for the population tested, we cannot estimate how large a decrease to expect. Should the value of the inbreeding coefficient in this population prove to be far from negligible, the fact that near 100% *H-2* heterozygosity occurs would mean that the effect of inbreeding is probably offset by heterozygous advantage at the *H-2* loci.

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Evolutionary nucleotide replacements in DNA

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With the increasing availability of analytical information on mRNA molecules, it is now possible to compare homologous nucleotide sequences from different organisms and to draw conclusions about their evolution. Such comparisons^{1,2} have shown that silent changes in codons occur more frequently than nucleotide replacements that produce changes in amino acid sequences (code-altering changes). Furthermore, there is an important difference between amino acid sequence comparisons and nucleotide sequence comparisons. The former show only differences in amino acid residues, but the latter show several types of differences when corresponding codons are compared³. Single-base replacements may be degenerate (silent) or expressed as amino acid replacements. Two-base codon changes may be degenerate, single-base changes, or be visible as such. Three-base codon changes may be degenerate (involving serine), simulate either single-base or two-base changes or be visible as such. All nine types of change are found in comparisons of genes from the viruses Φ X174 and G4. The relative numbers of these nine types as based on all possible interchanges between all 61 amino acid codons were listed by Holmquist *et al.*³ and are shown in Table 1. We discuss these results in the light of the significance of nucleotide changes in molecular evolution.

Theoretically, there are 526 possible single-base changes (substitutions) in the 61 codons for the 20 amino acids; 392 produce amino acid changes ('code-altering replacements') and 134 of the changes are silent. Changes that produce chain-terminating codons are omitted from this calculation, because such changes will be rejected. As a result, in a comparison of two homologous genes, one would expect to find on a random basis

25% of silent evolutionary single-base replacements. The calculations for codon changes involving two or three nucleotide replacements are complicated by the fact that such changes will often include more than one change at the same nucleotide site, some of which will be revertants³. A value much higher than 25% of silent replacements among single-base changes would show that code-altering changes are adopted more slowly than silent changes because of negative selection. The reverse finding would show evolutionary pressure against the adoption of silent changes as replacements. Table 1 shows that in three of the four summarised examples, code-altering single-base changes are 1.8–3.0 times as likely to be rejected as are silent changes. It could be suggested that silent changes are adopted rapidly because they are advantageous rather than neutral. However, advantageous changes can be incorporated only slowly during evolution because of substitutional load⁴, so this reason for the rapid adoption of silent changes is unlikely.

The fourth example consists of genes *B*, *E* and *K* of Φ X174 and G4. These are the three genes that are read as frameshifts in genes *A*, *C* and *D*. Barrell *et al.*⁵ and Godson *et al.*⁶ concluded that the gene *E* arose at a later stage of evolution than gene *D*. This would be consistent with the much lower percentage of silent changes in gene *E* (ref. 6). Such changes in gene *E* would nearly all produce amino acid changes in the predecessor gene *D*, because third positions of codons in gene *E* are first positions of codons in gene *D*. A similar reasoning can be applied to gene *K* as compared with its predecessors *A* and *C*, and to gene *B*, in which third codon positions are second positions of codons in gene *A* (refs. 5, 6). Therefore, silent changes in genes *B*, *K* and *E* would often be rejected as causing unacceptable amino acid changes in genes *A*, *C* and *D*. Indeed, a markedly low value for the per cent of silent changes might indicate *per se* that a gene is being read by a frameshift in a gene of earlier origin. The results in Table 1 show that with the predictable exceptions of genes *B*, *K* and *E*, the genes in phages Φ X174 and G4 have incorporated silent nucleotide replacements much more rapidly than code-altering replacements.

The question arises, are silent replacements actually neutral, or have they taken place for adaptive reasons, such as a requirement for a specific secondary structure in mRNA, or a preferential use for certain transfer RNAs in regulating the rate of synthesis of a protein? Modulation of the rate of translation by isoacceptor tRNAs, and adaptation of tRNA pattern to the demands of translation were among the reasons advanced by Kafatos *et al.*⁷ to explain the nonrandom use of synonymous codons in rabbit and human β -globin mRNAs. However, such arguments are unconvincing, for each β -globin mRNA has a different list of usage for synonymous codons. It seems highly improbable that closely related mammals would have different requirements for mRNA structure and tRNA usage for the same protein. If this were so, a staggering number of species differences, each necessitating a separate evolutionary pathway, would be needed at the molecular level for the thousands of different homologous proteins in each species. We therefore conclude that silent single-base replacements in the codons of β -globin mRNAs are probably neutral. This simple explanation probably also applies to the other comparisons of mRNAs in Table 1.

We have previously stated⁸ that 'if DNA divergence in evolution includes the random fixation of neutral mutations, then the third position nucleotides should change more rapidly, because synonymous mutations are more likely to be neutral'. This prediction agrees with the findings summarised in Table 1, with the exception, for reasons noted, of the 'frameshift genes'. Recently, Kimura¹ has drawn attention to the preponderance of silent replacements over code-altering replacements as evidence for the neutral theory of molecular evolution, based on comparisons of 53 nucleotide positions in human and rabbit β -globin mRNAs plus 84 positions in histone H4 mRNAs of two sea-urchin species. We have extended and expanded Kimura's findings, and Table 1 shows comparisons of 8,469 nucleotide positions.

Table 1 Evolutionary replacements in mRNAs and viral genes compared with interchanges in code

	Example	β -Globins	α - and β -Globins	Φ X174 vs G4 genes		Possible interchanges in code table
				In phase	Frameshift	
Single-base						
Degenerate	UUU/UUC	84	64	487	16	134
Recognisable	UUU/AUU	27	78	160	77	392
Per cent silent		76	45	75	17	25
Two-base						
Degenerate	CUC/UUA	2		31		28
Recorded as single-base	UUU/AUC	30	95	151	17	1,006
Recognisable	UUU/GAU	13	25	80	14	534
Three-base						
Degenerate	UCU/AGC			4		12
Recorded as single-base	UCU/GGC	1	2	29		308
Recorded as two-base	UCU/GUA	2	44	124	8	1,164
Recognisable	UUU/AAG		1	6		82
Totals						
Nucleotides compared		1,323	1,251	5,094	801	10,980
Nucleotide replacements		210	523	1,660	178	8,360
Silent replacements		122	207	894	41	3,012
Per cent silent replacements		58	40	54	23	36

The globin comparisons are of mRNA sequences from refs 7, 9, 10, 11 and 12. The β -globins are of rabbit, human and mouse and were compared pairwise. The α -globin was of rabbit, and was compared with the three β -globins. The gene sequences of Φ X174 and G4 are from refs. 5 and 6. The comparisons of the codon table are from ref. 3, and are the total of possible amino acid codon changes in both directions, excluding changes that involve chain-terminating codons.

It is obvious from results now available that the inaccurate and crude procedure of measuring evolutionary changes in terms of 'minimum base differences per codon' is defective and therefore obsolete.

We thank Dr J. C. Fiddes for supplying aligned comparisons of G4 and Φ X174 coding regions, and Dr Charles Weissmann for the unpublished sequence of mouse β -haemoglobin gene. We acknowledge support for T. H. J. by a grant from NASA. *Note added in proof:* Friedman *et al.*¹³ compared the sequences of the early regions of polyoma and SV40 genomes. Our examination of their Fig. 4 shows 65% silent single-base changes, and 43% silent replacements in 2,079 nucleotide comparisons with 1,080 replacements.

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Localisation of the γ -, α -, δ - and β -globin genes on the short arm of human chromosome 11

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Human-mouse somatic cell hybrids have proved invaluable in assigning human genes to their respective human chromosomes¹. To date, the success of this approach has depended on identifying human proteins which are synthesised in hybrid cells containing a small number of human chromosomes. Consequently, chromosome assignment has been limited mainly to human proteins which are expressed in

man-mouse somatic cell hybrids and for which a suitable assay, usually electrophoretic or immunological, exists to distinguish between the human and murine homologous proteins. This technique is therefore unsuitable for the assignment of those human genes which are expressed only in differentiated cells and not in hybrid cells. Here, we describe how nucleic acid hybridisation and restriction endonuclease mapping of DNA can be combined to test for the presence of human structural gene sequences within hybrid cell DNA. This method can be used to assign any purified human DNA sequence to a human chromosome, and does not require the DNA sequence to be expressed in man-mouse hybrid cells.

The genes that we have studied are those which code for the γ - and α -globin polypeptides of human fetal haemoglobin (HbF, $\alpha_2\gamma_2$ and $\alpha_2\alpha\gamma_2$) and those which code for the β - and δ -globins of major and minor adult haemoglobin (HbA, $\alpha_2\beta_2$ and HbA₂, $\alpha_2\delta_2$, respectively). DNA fragments containing the β - and δ -globin genes can be detected in restriction endonuclease digests of total human DNA by agarose gel electrophoresis of the digested DNA, transfer of the digest products to a nitrocellulose filter by blotting² and hybridisation of the filter with ³²P-labelled cloned complementary DNA (cDNA) made from human β -globin messenger RNA (plasmid pH β G1 (ref. 3)). The globin DNA fragments detected have been ordered into a physical map of restriction endonuclease cleavage sites around the human β - and δ -globin genes⁴. Similarly, cloned

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cDNA made from human γ -globin mRNA (plasmid pH γ G1 (ref. 3)) has been used as a nucleic acid hybridisation probe to construct a physical map around the γ - and α -globin genes⁵.

To assign these genes to a human chromosome, we therefore prepared DNA from a panel of human-mouse somatic cell hybrids, and digested the DNA with a restriction endonuclease which would release the β -related globin genes in DNA fragments of a convenient size for subsequent detection by electrophoresis and filter hybridisation with either 32 P-labelled pH β G1 DNA or pH γ G1 DNA. The restriction endonucleases chosen were *Pst*I, which liberates the β - and δ -globin genes in DNA fragments of 5.0 and 2.3 kilobase pairs, respectively⁴, and *Hind*III, which generates a 8.2-kilobase γ -globin DNA fragment and a 3.5-kilobase α -globin fragment⁶.

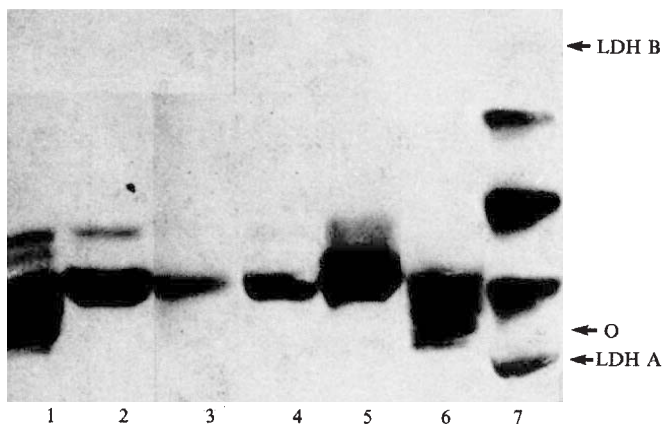


Fig. 1 Electrophoretic analysis of LDH in man-mouse somatic cell hybrids used in this study. Hybrid cell extracts were electrophoresed on cellulose acetate strips and stained for LDH activity as described elsewhere¹². Cells used were: (1) LBT.2; (2) mouse (3T3 cells); (3) LBT.2 grown in the presence of BUdR; (4) mouse (1R cells); (5) Cl.5; (6) LA.4; (7) human (HeLa cells). The electrophoresis origin (O) and positions of human LDH A and LDH B homotetramers are as indicated. Hybrids LA.4 and LBT.2 exhibit a multiple banded pattern characteristic of cells expressing human LDH A in addition to mouse LDH.

Early in this project we learned that Deisseroth *et al.* had used solution hybridisation of labelled human β - and γ -globin cDNA with man-mouse hybrid cell DNA to show that β - and γ -globin genes were located on human chromosome 11 (ref. 7). We therefore limited our investigation to DNA prepared from the four somatic cell hybrid clones described in Table 1. The hybrid LA.4 has been shown by karyotyping to contain human chromosomes 11 and X (ref. 8); as shown in Fig. 1, this hybrid cell line also expresses human lactate dehydrogenase A (LDH A) determined by the short arm of human chromosome 11 (ref. 9). A subclone of LA.4, termed Cl.5, had lost human chromosome 11 and therefore the ability to synthesise LDH A. A third hybrid clone, LBT.2, contained a human translocation chromosome comprised of the short arm of chromosome 11 and most of the long arm of chromosome 17 (11 cen $\rightarrow$ 11p15; 17q21 $\rightarrow$ 17qter)⁹. This chromosome enables LBT.2 cells to synthesise human LDH A and also codes for the soluble form of thymidine kinase (TK); a gene necessary for the expression of this enzyme has been assigned to the long arm of human chromosome 17 (ref. 10). The fourth cell line was derived by growing LBT.2 in bromodeoxyuridine (BUdR) to select against cells carrying the TK gene; this cell line, termed LBT.2-BUdR, had, as predicted, lost the human translocation chromosome and the ability to synthesise human LDH A.

DNA was prepared from each of these cell lines, digested with restriction endonucleases *Pst*I or *Hind*III and compared with restricted human and mouse DNA by agarose gel electrophoresis, transfer to a nitrocellulose filter and subsequent

hybridisation with 32 P-labelled pH β G1 or pH γ G1 DNA (Fig. 2). In the conditions of hybridisation used, each probe can readily detect its corresponding globin DNA fragments in 10 μ g digested human DNA. These human probes show little or no hybridisation to mouse DNA. In a reconstitution experiment in which human and mouse DNA were mixed in varying proportions before restriction and hybridisation, it can be seen that all β -related globin genes could be detected in human-mouse DNA mixtures containing as little as 10% of the normal level of human globin genes. Most important, γ -, α -, δ - and β -globin DNA fragments were clearly detectable in hybrid cell lines LA.4 and LBT.2, which contain human chromosome 11 and the short arm of 11, respectively, and were absent in DNA prepared from Cl.5 and LBT.2-BUdR cell lines, neither of which contain chromosome 11. This experiment confirms the assignment by Deisseroth *et al.* of the human γ - and β -globin genes to human chromosome 11 (ref. 7). It also demonstrates that all four β -related globin genes are located within the short arm of chromosome 11 within the region 11 cen $\rightarrow$ 11p15 (ref. 9). This assignment is formally established by the segregation of the globin genes during selection against the linked TK marker in LBT.2 cells grown in the presence of BUdR.

Table 1 Man-mouse somatic cell hybrids used in the present study

Hybrid cell line	Human chromosomes identified	Human LDH A	β -Related human globin DNA fragments
LA.4	11, X	+	+
Cl.5	X	-	-
LBT.2	t(11:17)(p15;q21)	+	+
LBT.2-BUdR	-	-	-

Hybrid cell lines LA.4, Cl.5 and LBT.2 were grown in RPMI 1640 medium supplemented with 100 μ M hypoxanthine, 10 μ M methotrexate and 16 μ M thymidine. LBT.2-BUdR was obtained by culturing LBT.2 cells for 12 transfers in RPMI 1640 medium supplemented with 30 μ g ml⁻¹ BUdR. The human chromosome constitution of the cell lines was confirmed by Giemsa 11 and trypsin banding analysis of metaphase preparations (M. Bobrow, personal communication). >90% of LBT.2 cells contained the human translocation chromosome. Detailed quantitative estimates of proportions of LA.4 and Cl.5 cells which contain the indicated human chromosomes are not available. The presence or absence of human LDH A was assayed by electrophoresis of hybrid cell extracts on cellulose acetate (Fig. 1). The analysis of β -related human globin DNA fragments is described in Fig. 2 legend.

This approach to assigning human genes to human chromosomes is simple, fast and only requires that a suitable purified DNA be available as a nucleic acid hybridisation probe. It is also sensitive and enough DNA can be isolated from 10⁷ man-mouse hybrid cells to test for the absence or presence of structural gene sequences. In this study the assignment was aided by a very low level of cross-hybridisation between the human globin-gene probes and mouse DNA in the hybridisation conditions used. However, this approach could also be extended to probes which hybridise equally well to both human and mouse DNA, and only requires a restriction endonuclease which generates different electrophoretic patterns of hybridisable DNA fragments from human and mouse DNA. With the advent of libraries of cloned human DNA fragments¹¹, this assignment procedure could be used to identify cloned DNA fragments from any required human chromosome.

The assignment of human genes to chromosomes by analysing DNA sequences has two major advantages over the alternative approach of studying the production of human proteins in man-mouse somatic cell hybrids. First, it does not require expression of human genes and is therefore suitable for the assignment of genes coding for proteins such as globins which

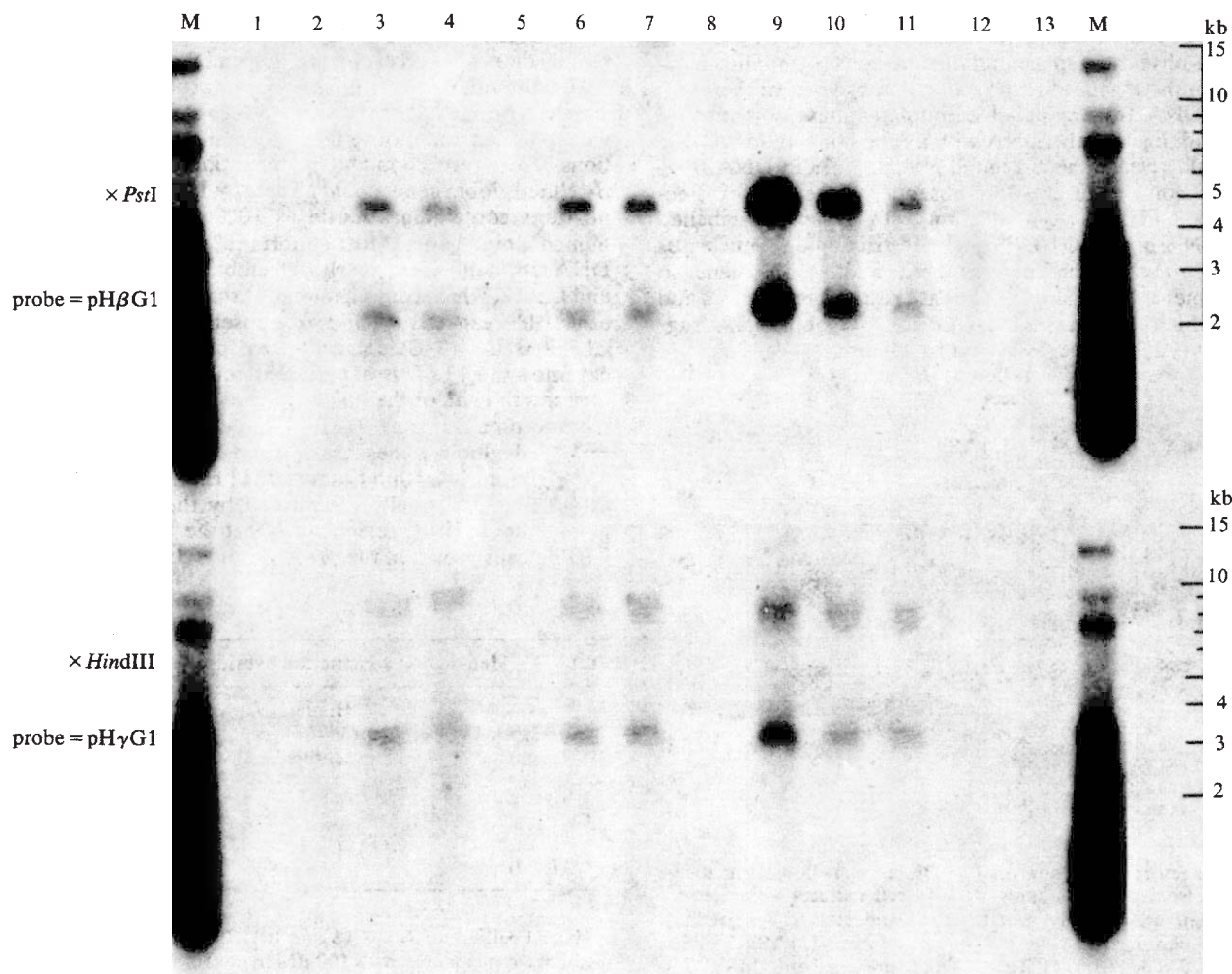


Fig. 2 Analysis of human β -related globin DNA fragments present in restriction endonuclease digests of man-mouse somatic cell hybrid DNA. DNA was prepared from human placenta, from BALB/c mouse brain, from cultured 1R cells and from four man-mouse somatic cell hybrids, using methods described elsewhere¹³. Batches (10 μ g) of DNA were digested with endonuclease *Pst*I or *Hind*III (Microbiological Research Establishment, Porton Down). DNA digests were recovered, alkali-denatured, electrophoresed through a 1% agarose slab gel and transferred by blotting to a nitrocellulose filter. pH β G1 and pH γ G1 DNA were labelled with 32 P *in vitro* by nick translation with [α - 32 P]dCTP (2,000–3,000 Ci mmol⁻¹, Amersham) to a specific activity of 1.5×10^8 d.p.m. per μ g DNA. After pretreatment of the filters to prevent nonspecific binding of single-stranded DNA, the filters were hybridised for 2 d at 65 °C in 1 $\times$ SSC (saline sodium citrate; 0.15 M NaCl, 15 mM trisodium citrate (pH 7.0)) with 30 ng ml⁻¹ heat-denatured labelled DNA. Unbound labelled DNA was then removed by washing at 65 °C in 1 $\times$ SSC and nonspecific DNA hybrids removed by a stringent wash in 0.1 $\times$ SSC at 65 °C. Labelled DNA bands were detected by exposing the filters to X-ray film for 15 d in the presence of an intensifying screen. Full details of the preparation of DNA, restriction endonuclease digestion, gel electrophoresis, DNA transfer, nick translation, filter hybridisation, washing and autoradiography are described elsewhere¹³. Samples electrophoresed were: (1) BALB/c DNA; (2) 1R DNA; (3), (4) separate preparations of LA.4 DNA; (5) Cl.5 DNA; (6), (7) separate preparations of LBT.2 DNA; (8) LTB.2-BUdR DNA; (9) human DNA; (10) 1:1 human: BALB/c DNA; (11) 1:3 human: BALB/c DNA; (12) 1:9 human: BALB/c DNA; (13) BALB/c DNA. The hybridisation markers (M) consisted of 10 pg pCRI DNA $\times$ *Eco*RI (13.3-kilobase fragments), 10 pg pCRI DNA $\times$ *Kpn*I $\times$ *Hind*II (7.3, 1.3, 0.93, 0.86, 0.64, 0.18, <0.1-kilobase fragments) plus 10 pg pCRI DNA $\times$ *Eco*RI $\times$ *Hind*III (9.8, 3.4-kilobase fragments); pCRI is the plasmid vector used in constructing pH β G1 and pH γ G1 (ref. 3).

are only produced by differentiated cells. Second, in hybrid cells in which both human and mouse homologous proteins are being synthesised, assignment depends on 'distinguishing between the two species' proteins. If the species difference is solely due to differences in the primary amino acid sequence of the proteins, then assignment will lead to the chromosome localisation of the protein's structural gene. On the other hand, if the difference reflects a species-specific difference in, for example, post-synthetic modification of otherwise identical human and mouse proteins, then analysis of man-mouse somatic cell hybrids could result in the assignment, not of the structural genes for the protein, but of the human modification system instead. Direct analysis of DNA sequences and structural genes in the nuclear DNA of somatic cell hybrids avoids this ambiguity and leads directly to the assignment of the structural gene.

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matters arising

Sm-Nd systematics of Lewisian gneisses and the origin of granulites

THE demonstration by Janardhan *et al.*¹ of the purging of H₂O from previously hydrous hornblende-biotite gneisses by CO₂-rich volatiles of possible mantle origin to form pyroxene gneisses is an important development in the study of deep crustal materials. A popular model to explain the chemical peculiarities of granulite facies gneisses (depletion in K, Rb, Th and U; high K/Rb ratios and low Rb/Sr ratios) has been degassing of their precursors during high grade metamorphism²⁻⁵. The probability of this having been initiated by increase in p_{CO_2} has been recognised by Tarney and Windley⁵. The degassing model received significant support from Nd isotope studies of the Lewisian gneisses from Scotland⁶. There, Sm-Nd dating revealed an age ($2,920 \pm 50$ Myr) of primary separation from the mantle some 200 Myr older than that of chemical differentiation (loss of Rb and U in particular) recorded by whole rock Rb-Sr and Pb-Pb and zircon U-Pb methods. Hamilton *et al.*⁶ imply that the repository for Rb expelled from the granulite facies Lewisian is, in part, the tectonically higher amphibolite facies component of that complex, which now has an Rb/Sr ratio that is far higher than is compatible with their ⁸⁷Sr/⁸⁶Sr ratio at 2,670 Myr ago⁷.

There are important geochemical differences between the granulite and amphibolite facies components of the Lewisian complex that are not discussed by Hamilton *et al.*⁶ and to which the discovery of Janardhan *et al.* is relevant. The pyroxene gneisses in the Lewisian of the isles of Tiree and Barra have positive Eu anomalies and abnormally high Sr (average, 837 p.p.m.) whereas tectonically higher hornblende-biotite gneisses have negative Eu anomalies and much less Sr (average, 381 p.p.m.) (refs 4, 9 and unpublished data). Both may be features of a primary igneous fractionation of the gneiss precursors, the pyroxene gneisses representing crystallisation under low $p_{\text{H}_2\text{O}}$ of a precursor tonalitic magma, the hornblende-biotite gneisses the ex-

pelled, residual, hydrous magma^{8,9}. The complementary patterns could not reflect simple loss of a hydrous vapour phase from the granulites, but could reflect the addition of Sr and Eu to more normal precursors of the pyroxene gneisses from a mantle-derived vapour phase⁴, such as that indicated by Janardhan *et al.*¹.

The dilemma is first that a model involving primary igneous fractionation at low $p_{\text{H}_2\text{O}}$ (implying high p_{CO_2}) of the pyroxene gneisses can as equally account for the K, Rb, Th and U depleted nature of the Lewisian pyroxene gneisses as for their high Sr and positive Eu anomalies⁹, thereby making degassing unnecessary as a means of chemical fractionation. Second, that addition of Sr, Eu and presumably other rare earth elements (REEs) from mantle depths in a CO₂ rich vapour phase would have a significant effect on Sr and Nd isotope systems in pyroxene gneisses.

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HAMILTON *ET AL.* REPLY—There may be differences in Sr content between granulite and amphibolite facies gneisses in the Scottish Hebridean Islands, but in the much more extensive outcrop on the mainland, Tarney (ref. 1, Table 1), using a much larger sample population, found only a small difference (615 as against 580 p.p.m. Sr) in gneisses of equivalent major element composition. Moreover, studies of REE distributions in the granulites^{2,3} show that it is the more frac-

tionated siliceous gneisses which have the more prominent positive Eu anomalies. The rare earth relationships are very similar to those described recently by Arth *et al.*⁴ in a Proterozoic gabbro-diorite-tonalite-trondhjemite plutonic suite from South-West Finland, interpreted as resulting from 'wet' hornblende fractionation. Thus they indicate the opposite petrogenetic relationship from that suggested by Drury⁵, but are entirely consistent with the isotope systematics which indicate that the dry granulite facies metamorphism reached its peak some 100–200 Myr after the gneisses were formed. This is also consistent with the conclusions of Janardhan *et al.*⁶.

With regard to Drury's dilemma, we can only point out that both granulite facies and amphibolite facies samples define the $2,920 \pm 50$ Myr Nd isochron. Had there been extensive addition of Sm, Nd and other REEs to the granulite facies gneisses, it seems to us most unlikely that the observed Sm/Nd–¹⁴³Nd/¹⁴⁴Nd correlation would have been maintained. Thus, until we see firm evidence to the contrary, we consider that the normally stable REEs have remained relatively immobile during granulite facies metamorphism, while the commonly mobile elements K, Rb, Th and U have indeed been partially removed.

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A model for the preferential degassing of the upper mantle

CRITICAL to the model of the depletion of the mantle presented by Hart *et al.*¹ is the assumption that $3.2 \times 10^{-6} \text{ ml g}^{-1}$ is an upper limit to the concentration of ^{40}Ar in the mantle source of submarine basalts. This value was obtained from the maximum slope of the upper envelope of data on a plot of $^{40}\text{Ar}/^{36}\text{Ar}$ against $1/^{36}\text{Ar}$. This interpretation ignores the probable significant depletion of initial magma Ar by strong partitioning into CO_2 bubbles and their loss before and during emplacement of magma²⁻⁴. Further, it has been demonstrated that natural cracks as well as those produced during sample preparation will probably release a considerable part of the CO_2 in vesicles, whose existence has been recently confirmed⁵. Superimposed on this alteration of mantle ^{40}Ar is the variable contamination with atmospheric Ar^3 . Thus the simple interpretation based on the upper envelope of scattered points seems to be invalid. Further, a basic contradiction follows if we apply their value to a limiting model requiring coherency of ^{40}Ar with K transfer; this coherency must apply in their model to the formation of oceanic crust at the ridges as this is the source of the degassed Ar as well as the transfer of K to the sialic crust (see Fig. 1 of Hart *et al.*¹). Their derived maximum ^{40}Ar concentration of the mantle source of submarine basalts is a factor of 0.04 lower than the coherent ^{40}Ar concentration computed assuming a K concentration of 0.1%. The coherent concentration represents the ^{40}Ar produced from this K concentration in 4,550 Myr. As I pointed out previously², this does not require a single-stage mantle history, only coherent degassing at each melting event. Self-consistency demands use of a computed ^{40}Ar concentration of about $8 \times 10^{-5} \text{ ml g}^{-1}$ for the initial magma in a limiting coherent model. Correcting for enrichment on partial melting gives an ^{40}Ar concentration of $2 \times 10^{-5} \text{ ml g}^{-1}$ for the depleted upper mantle source. Application of this to an estimate of the K concentration of the non-depleted mantle (their Fig. 3) leads to values of $>300 \text{ p.p.m.}$ even for very high extent of depletion of the mantle. Their other arguments are not qualitatively changed.

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HART *ET AL.* REPLY—The concentration of ^{40}Ar in submarine basalts is not critical to the principal observation made in our model calculation of preferential mantle degassing¹. The main point we stress is the similarity in the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the atmosphere and non-depleted lower mantle if the atmosphere and sialic crust formed preferentially as a coherent system from the upper mantle. We chose to estimate the ^{40}Ar content of the depleted upper mantle from the ^{40}Ar content of submarine basalts rather than to calculate it indirectly from the K content of the sialic crust and mantle because these values are not well established.

Schwartzman's value of $2 \times 10^{-5} \text{ ml g}^{-1}$ for the ^{40}Ar argon content of the mantle is only slightly different from our value of $4.5 \times 10^{-5} \text{ ml g}^{-1}$ calculated for the non-depleted lower mantle. Adoption of his calculated value would enhance the similarity in the $^{40}\text{Ar}/^{36}\text{Ar}$ ratios of the atmosphere and non-depleted mantle. The concentration of ^{40}Ar measured in submarine basalts is at least an order of magnitude lower than these calculated values. We suggest that this is evidence for an upper mantle depleted in ^{40}Ar relative to the deep mantle and is consistent with the well-established depletion of K in the mantle source of ridge basalt.

There is no evidence that Schwartzman's 'probable significant' degassing of the basalt magma under oceanic ridges by CO_2 bubble transfer is more significant than degassing by basalt-seawater interactions after magma emplacement or than degassing during partial melting and metamorphism in subduction zones and within continental cratons. Because Si and Ca are not partitioned into residual phases

or CO_2 bubbles during magmatic processes, the high flux of these elements and ^3He observed in geothermal plumes from the Galápagos Spreading Center² suggests basalt-seawater interactions rather than bubble transfer are the dominant mechanism for degassing noble gases in ridge basalts today.

We have extensively discussed the effects of atmospheric contamination and degassing on the concentration of ^{40}Ar in submarine basalts and suggest that the surprisingly uniform concentration rules out significant degassing by CO_2 bubble transfer before magma emplacement^{3,4}. The ^{40}Ar contents of basalts recovered from depths of over 5,000 m in the Cayman Trough are very similar to those of typical ridge basalts suggesting the ridge basalts have not degassed more than basalts extruded in deep trenches. Basalts with well developed vesicles that have obviously undergone extensive degassing by bubble transfer have been dredged from shallower portions of the ocean and have ^{40}Ar contents up to an order of magnitude lower than the maximum of $3.2 \times 10^{-6} \text{ ml g}^{-1}$ we adopted for our model.

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The late-Würm Arctic ice sheet

I HAVE recently realised that we did not give Dr John Mercer full credit for his earlier contributions when we wrote our paper *Was there a late-Würm Arctic Ice Sheet?*¹. In our reference to Mercer's paper *A former ice sheet in the Arctic Ocean?*² we should have noted that he was the first to make a mass balance calculation for Arctic ice shelves based on present snowfall over the Arctic Ocean and present iceberg calving rates of Antarctic ice shelves; he had also postulated that late-Würm Arctic ice shelves may have occupied the northern Norwegian Sea; and he had also commented on the evidence for ice from the Arctic Ocean moving southward onto the north slope of Alaska. Mercer's (1970) comments on an ice shelf in the Norwegian Sea were a summary of ideas he had developed more fully previously³.

Our 1977 paper in *Nature* differed from Mercer's (1969 and 1970) papers in that it developed the idea that ice shelves in the Arctic Ocean, Norwegian, Greenland, and Labrador Seas, and Baffin Bay would

have been components of a late Würm Arctic Ice Sheet that also included the Laurentide, Innuitian, Greenland, Barents-Kara Sea, British, Scandinavian, and possibly East Siberian Sea grounded ice sheets. This connection is implicit, but not explicit, in Mercer's work. We treated the grounded and floating ice complex as a single, unified dynamic system. As a result, our mass balance calculation included not only the Arctic Ocean (which Mercer treated), but also snowfall over the portions of these fringing ice sheets which drained into the Arctic Ocean and its adjacent seas and bays, and distributed that input along an ice-shelf calving front much further south than Mercer's.

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reviews

Diamond facets

John Walker

The Properties of Diamond. Edited by J.E. Field. Pp. 674. (Academic: London and New York, 1979.) £32.50; \$67.25.

THIS book could reasonably be subtitled "The Special Research Project: A Second Progress Report", for it chronicles the advances made in diamond research since *The Physical Properties of Diamond* was published in 1965 (Clarendon: Oxford). (This latter should have been subtitled "A First Progress Report", of course.) The Special Research Project was established in 1949 by Sir Ernest Oppenheimer of de

developments; they would also bring to bear different ideas and perspectives on current problems in diamond research. There may be difficulties in including contributions from them, but there are precedents.

An integral part of the Special Research Project is the annual Diamond Conference, memorable features of which are the quotations it produces. Dr Field reproduces two favourites. I would like to add two more: Q. "What happens at higher temperatures?" A. "The apparatus melts" (H.M. Rosenberg); "As work in the de Beers Laboratory has shown, the de Beers grit is best" (D. Tabor).

To return to the book, it contains twenty chapters, each written by a different author (or authors), under eight headings: solid state (thermal, optical and electronic properties and impurity content), theoretical, surface (adsorbability and optical properties), mechanical (strength, friction, abrasion, indentation, effects of high temperature, internal structure), physics and technology of growth, geology (of the diamond itself and of inclusions within the diamond) and industrial (abrasive and non-abrasive uses). Finally there is a 12 page appendix which tabulates the physical and chemical properties of diamond, including references to the original research.

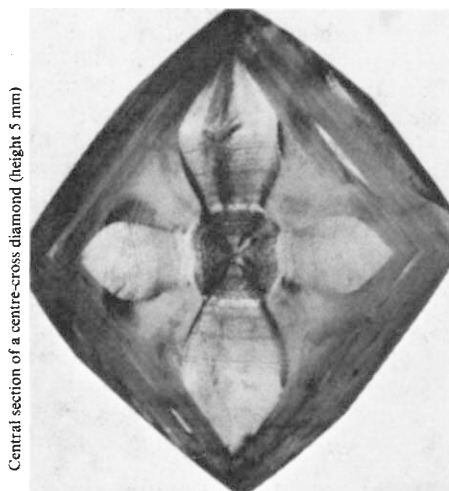
The chapter on non-abrasive uses of diamond may contain surprises, even for the diamond specialist. We all know about gemstones, and about cutting and grinding uses. Other uses include: windows (small ones of course!) in satellites; high pressure cells in scientific experiments; microtome knives for eye and brain operations; radiation detectors, especially in radiotherapy; thermometers; and as heatsinks for semiconductor devices.

Although it is unlikely that diamonds will become cheap and plentiful, two chapters in the book suggest potential new sources. While Professor Dawson must be gratified that diamonds are now being found in Australia, the late Professor Tolansky's predictions of diamonds on the moon has yet to be verified. (An interesting statistic is that Tolansky inspected 10 million individual diamonds during his studies — he professed to do it as an alternative to watching television!)

Although Professor Sellschop and his team cannot claim to have investigated so many specimens, they report some fascinating results in their activation analysis studies. The impurity content of

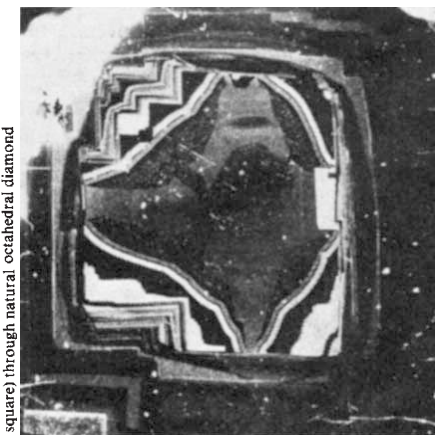
diamond has long been a topic of interest, and this new technique has provided data of remarkable quality and quantity — particularly on the submicroscopic inclusions which seem to be droplets of the magma from which the diamonds crystallised. The extension of these nuclear methods to the light elements, which so far have been less intensively investigated, is eagerly awaited, as the light elements are more likely to be able to enter the diamond lattice itself.

Another new technique — cathodoluminescence — is providing a wealth of



Central section of a centre-cross diamond (height 5 mm)

From Suzuki and Lang, 1976



Optical micrograph of etched near-central section (2.4 mm square) through natural octahedral diamond

From Harrison and Tolansky, *Proc. R. Soc.*, 1964

Beers and Sir Francis Simon of the Clarendon Laboratory to encourage cooperation in fundamental research between the Diamond Research Laboratory and various British universities (and a very fruitful arrangement it has been).

What the book lacks in my opinion, and what my subtitles were intended to clarify, is a contribution from Soviet and American scientists. Both of these groups have made considerable advances in pure and applied diamond research. American scientists were the first to publish, in the pages of this journal, a report of the successful synthesis of diamond (Bundy *et al.*, *Nature*, **176**, 51-55; 1955). (Indeed the Americans can not only synthesise diamond; they can do it using peanut butter as the starting material — a valuable discovery if ever the EEC establishes a peanut butter mountain.)

This is not to say that the book does not give credit where credit is due — indeed it does — there are copious references to US and Soviet papers. My point is that the American and Russian authors are in a better position to chronicle their own research, especially the latest

data about point defects (and linear and planar ones too), as well as elucidating the internal structure of diamonds; it is a valuable complement to X-ray and optical transmission techniques. And one of its practitioners, Dr Lang, seems likely to replace Professor Tolansky as a supplier of beautiful photographs of diamond.

It is impossible in the space available to consider in detail all 20 chapters of the book; suffice it to say that the authors are leading lights in their fields.

One of the surprising things about diamond — which in principle is one of the simplest solids one could envisage — is the wealth of studies it has generated, and the continuing exhibition of new and interesting features. Yet we still do not know how it was generated by nature, nor do we understand its frictional behaviour, despite the manifest importance of such topics. But we are trying hard, as the 674 pages of this authoritative book indicate; this is where a scientist must start if he is interested in any facet of diamond. □

John Walker was a Research Fellow at the Universities of Reading and Paris, studying the optical properties of diamond. He is now a microprocessor consultant.

Earthquake phenomena

The Earthquake Handbook. By Peter Verney. Pp. 224. (Paddington: New York and London, 1979). £5.50.

The Earthquake Handbook is an excellent description of earthquake phenomena and fulfills the objectives set out by the author in the introduction: "This is not a technical book: those wanting detailed scientific information [on earthquakes] should consult some of the many excellent works available . . . My interest has been to explain this extraordinary natural phenomenon, referring to the records of past earthquakes, and in the light of modern progress in seismology, to help those who may live in or visit one of the many earthquake-prone areas of the world". The extensive quotations from eye-witnesses to the earthquakes described and the opinions of their contemporaries are certainly the outstanding features in this book, but the author's own contributions are clear and concise. The book is illustrated by an excellent selection of photographs, prints and drawings, all well reproduced. The text, figures, and quotations taken together give as good a description of what an earthquake is really like as any other source short of the earthquake experience itself. However, it should be understood that the treatment is descriptive and the technical aspects of seismology are scarcely touched on. In this sense the title of the book may have been poorly chosen.

The book begins with descriptions of the 1906 San Francisco and 1755 Lisbon earthquakes. These descriptions are amplified in subsequent sections of the book, together with a description of the 1923 Tokyo earthquake based principally upon the eye-witness account of H.W. Kinney, an author and editor who was travelling near Tokyo at the time. Shorter descriptions of the earthquakes at Port Royal, Jamaica (1692), London (1750), Calabria (1783), Messina (1908), Agadir (1960), Skopje (1963) and Alaska (1964) are also included.

The book then gives a very brief account of the various earthquake theories from the earliest mythologies (which generally attributed earthquakes to movement of the animal that was thought to support the Earth) to Aristotle (winds blowing through subterranean passages), Lucretius (rocks displaced in subterranean caverns), Ben Franklin (an electric discharge from a cloud to Earth), and John Michell in 1760 ("earthquakes were waves set up by the shifting masses of rock miles below the surface"). Professor Koto of Japan in 1891 was apparently the first to assert that faulting was the cause (and

not the effect) of the earthquake. Verney, of course, quotes extensively from those who attribute earthquakes to divine punishment (for example, Royal Society, 1752, "earthquakes only occur when people need chastening"). The section concludes with an elementary description of plate tectonics.

A description of earthquake geography and earthquake effects follows. The latter section is particularly concerned with tsunami (tidal waves generated by earthquakes) but includes discussions of earthquake noises and lights as well as the physiological shock experienced by earthquake survivors and even the motion sickness reported by some.

The basic rules for survival in an earthquake are given in a brief but

practical chapter that includes advice on how to prepare for a possible earthquake as well as how to behave during and after the shock. The final three chapters are brief, non-technical treatments of earthquake engineering, prediction, and control. I believe most scientists will find these final three chapters somewhat superficial.

The book is clearly intended for a lay audience, but I believe even seismologists will find the many quotations and general descriptions of real interest. However, the prospective reader is reminded of the author's admonition "This is not a technical book . . .". **James C. Savage**

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Nutrition and national development

Food and Nutrition Policy in a Changing World. By J. Mayer and J. Dwyer. Pp.300. (Oxford University Press: Oxford and New York, 1979.) £8.25.

LIKE many books which are edited rather than authored this slim volume seems to me to lack the sense of coherence and direction that would seem a *sine qua non* for it to achieve its aim of discussing the need for placing food and nutrition in the larger context of national development. This failing is doubly said, as it is not merely a book, but a condensation of a five-volume report commissioned from the editors by UNICEF in order to "set forth goals for UNICEF and allied agencies for the next decade".

What use the report will be to UNICEF, only time will tell; I found the book unsatisfactory to read. Partly this was no doubt due to the level at which it was pitched, for as the editors warn: "The specialist would regard the book as nontechnical and somewhat cursory". But it was more than that: it was a sense that it was not a book at all but a collection of disparate chapters loosely edited and fitting together into a scheme which seemed only to exist as the various subheadings in the table of contents.

This is not to say that some of the individual chapters were not quite enjoyable as discrete essays: the editors themselves contributed a chapter entitled "Beyond Economics and Nutrition: The Complex Basis of Food Policy" which was a reprint of their polished critique of approaches to control of malnutrition, first published in *Science*. Some other chapters, notably those by Perisse and Ms Jeliffe, were also well written expositions of the views of the authors. But

in this I found the flaw with the book as a whole. Each author seemed to perform a solo item, without reference to his colleagues so that overall the book did not hang together.

I was irritated by such contradictions as Kimm and Donoso repudiating the idea of dietary protein deficiency on page 160, while Winikoff invoked it *en passant* on page 184; or again that Winikoff's chapter advocating megadoses of vitamin A as an effective treatment for vitamin A deficiency was followed by an editorial note pointing out it was a high risk and probably ineffective strategy. Besides such tactical differences, there seemed also to be a difference in grand strategy between the advocates of economic intervention and those who felt that health care was specifically needed.

In general, I would not criticise any scientific work for the divergencies in views it contains. After all, without contradictions there is no science; the great mistakes in nutrition have arisen from professional certainty, not doubt. But the differences in view in this book are not productive ones. There is no air of creative tension between them; on the contrary, the overwhelming impression is that the authors have not even discussed their views with each other. I do not know why this has arisen, I suspect because of a lack of firm editorial control at the centre, and perhaps for some authors a lack of balanced scholarship in the area which they seek to discuss, but it is difficult to judge the extent of scholarship in the space allotted to each author. The editors would have done better to throw out some topics completely (for example, Dr Araujo's ludicrously terse "Nutrition Primer") and enlarge other issues in depth. But they did not, and I suspect the result is a book that will not interest the layman or satisfy the specialist.

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HLA system

The HLA System: An Introductory Survey. Second edition. By A. Svejgaard, M. Hauge, C. Jersild, P. Platz, L.P. Ryder, L. Staub Nielsen and M. Thomsen. Pp. 111. (S. Karger: Basel, London and New York, 1979.) DM 43; \$21.75; SFr. 36.

THE HLA system of tissue antigens is acquiring a growing importance in the study of cellular interactions. A coherent, lucid account such as this volume attempts is to be welcomed. The small size of the book (111 pages) in no way reflects a paucity of information. The authors have achieved the combination of brevity and clarity, at the same time providing useful links for readers who may come to the subject from disciplines which do not encompass genetics or human disease.

The scope of work now relevant to HLA is indicated by the contents page, with topics ranging from immune response (Ir) genes to complement, transplantation, transfusion, pregnancy, paternity testing and disease association. The bibliography is comprehensive and commendably up to date. There is a simple but adequate account of the biology of the HLA system,

with particular reference to the role of the major histocompatibility complex (MHC) in the immune response. This section also includes a summary of current thinking about the specific role of HLA antigens as cell membrane products (? receptors).

There is a good appraisal of the current status of HLA in clinical medicine, including both transplant matching and disease association studies. The authors have summarised these two major applications and give a very honest view of the advantages and disadvantages of applying HLA studies in these areas. There is a short concluding section on methods in HLA typing which is more than adequate for those who may not work directly on HLA but need to know about the general techniques.

This book could be required reading for all non-HLA workers who are proposing to collaborate on HLA studies with the experts in the field. It would save a great deal of time presently spent on lecturing on HLA to various groups of medical and scientific workers who are looking for a simple, understandable introduction to HLA.

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Physics of neutron stars

Neutron Stars. By J.M. Irvine. Pp.155. (Oxford University Press: Oxford, 1978.) £7.95.

IRVINE'S monograph is the first book devoted to the physics of neutron stars, the second most extreme endpoint of stellar evolution. The primary content is an informal review of the theory of neutron star structure, with emphasis on our understanding of the physical processes in condensed matter that is needed in order to construct 'realistic' models.

The book begins with a broad outline of stellar evolution, and the mass-radius relationship of neutron stars is estimated in the manner familiar to students of stellar interiors since the work of Eddington. The basic observation of pulses from isolated pulsars is then briefly described, followed by a repetition of the now classic arguments for the identification of pulsars as rotating neutron stars, based on their rate of increase of pulsar periods and the shortness of the period of the Crab pulsar. (The use of the Crab nebula as a box calorimeter to measure the moment of inertia of the central object, an equally crucial part of the identification, is not mentioned.) This is followed by a fairly detailed discussion of glitches in the spindown of the Crab and Vela pulsars along with a review of the

starquake model. No mention is made of the 'restless' random walk of the rotation frequency of the Crab pulsar, which is probably also present in the Vela and many other pulsars.

From here, Irvine turns to an outline of cooling processes that can influence the final temperature of a neutron star, both in the deep interior and at the surface. He does a reasonably good job of outlining the significance of superfluidity, superconductivity and the formation of a pion condensate for the cooling rates, a topic which is rapidly increasing in experimental importance as the HEAO-2 observing programme moves forward. An understanding of his discussion requires at least slight acquaintance with the BCS theory of superconductivity. He then turns, in chapter 4, to a brief discussion of neutron star 'exteriors'. Here, the magnetosphere is treated solely as a source of vacuum magnetic dipole radiation (including the ~ 20% relativistic corrections!), in spite of his mentioning the requirement of there being an electrically significant number of charges in the environs. Irvine then returns to the less controversial realm of condensed matter by describing the peculiar atoms and solids which make up the perfect lattice (density ~ 10^4 g cm³) expected to form the very outermost layers. This region (often called 'Ruderman's whiskers') is treated with about the same depth but less clarity as is contained in Ruderman's discussion in the

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IAU Symposium No. 53 (D. Reidel: Dordrecht, 1974, p117). A few remarks are made on possible radiation of pulsed photons by charges accelerated along polar field lines, including some incorrect remarks on the formation of purely radio pulses because of synchrotron self-absorption of higher emitted frequencies.

The topic of observational appearance is then rapidly abandoned for a return to the interior, with a brief outline of the equation of state including various possibilities in the very high density region (pion condensation, hadronic excited states and cores, and "quark soup"). The application of these equations of state to the calculation of stellar models and the maximum and minimum masses of neutron stars is then outlined, and the book concludes with three brief appendices on methods for calculating the correlations which give rise to superfluidity, as well as a few results from general relativity needed to obtain the 20–30% corrections of Newtonian theory expected for these objects.

Although this book will be mildly useful to specialists in neutron star physics (and somewhat less than mildly useful to those concerned with pulsars as radiating bodies), it suffers from a number of serious problems. It is very badly organised; topics in condensed matter physics are interspersed with astronomical issues (such as scintillation of pulsar radio signals) and with occasional excursions into rudimentary discussion of the magnetosphere. Apparently, the text was written before 1976 and has not been substantially updated; for example, the thick crust neutron stars of Pandharipande, Pines and Smith are not mentioned, and pulsed accreting X-ray sources do not appear at all. This latter omission is especially astonishing, as our real laboratory of neutron star interiors is likely to be the accreting objects in binary systems, not the "... vast amount of information ... buried in the glitch phenomena ..." of isolated radio pulsars.

Irvine's discussion of the coupling of the isolated neutron star to the outer world is so perfunctory that its omission would have improved the book. For example, I find it difficult to understand the inclusion of the calculation of vacuum dipole radiation in curved spacetime (a 20% correction), while no illustration is given of the orders of magnitude uncertainty introduced by our lack of understanding of the exterior plasma configuration. His discussion of how pulses form is wholly out of touch with modern ideas; however, given the lack of contact between these ideas and the observational details, this topic would also have been better omitted, or dealt with in sufficient depth to illustrate the nature of the problem. His discussion of the interior physics is adequate for the expert, but inadequate as an educational tool for those

not already acquainted with theoretical many body physics.

As a reference source, the book is incomplete, and not more up to date than the thorough review by Baym and Pethick (*A. Rev. nucl. Sci.*, **25**, 27; 1975). The flavour of *Neutron Stars* is that of unrevised, rather outdated lecture notes

for an advanced course, without the compensation of new ideas or a unified point of view. A monograph which is truly useful remains to be written. **J. Arons**

J. Arons is Professor of Astronomy at the Leuschner Observatory, University of California at Berkeley.

Individual and group differences

The Psychology of Individual and Group Differences. By L. Willerman. Pp.531. (Freeman: San Francisco and Reading, UK, 1979.) Hardback \$15; £8.70.

Readings About Individual and Group Differences. Edited by L. Willerman and R.G. Turner. Pp.330. (Freeman: San Francisco and Reading, UK, 1979.) Hardback \$14; £8.90; paperback \$6.50; £3.80.

THE analysis of individual and group differences, notably those connected with one form or another of ability, has become enmeshed in the confusions of political ideology. Theories that human talents may be inherited, or even that there are human talents, have fallen under a threat of popular disapproval divorced from the scrutiny of evidence or of academic discourse. Given the intolerant tyrannies of the past it is not so hard to interpret the censorship of Copernicus or the curtain drawn over the free development of genetics in Stalin's Russia. But this new censorship, arising from outside the formal apparatus of the state, is more difficult to account for, although in character and effect it is no less insidious. As a consequence, in recent times, academic thinkers willing to argue for the inheritance of intellectual skills have been subjected to strong attacks, often of an unreasoned kind, and their views have been identified with common prejudice. It has sometimes required an act of courage to permit open consideration of the empirical evidence.

The author of this new text on the psychology of individual and group differences pays scant attention to such distractions, and leads the reader straight into the heart of his subject. His report is directed unambiguously to a hierarchical conception of difference. The first chapter deals with the academic background of research, wholly ignoring political overtones or implications. The next two cover some basic elements of statistics and genetics thought necessary for an understanding of the later sections. Inevitably these are brief and probably too difficult for the complete beginner in either discipline, but they do serve as a tribute to the need to master such attendant skills. If no more, they alert the reader to think seriously about the methodological problems that have to be tackled in psychological research.

In the second and major section of the book, the author works patiently through some of the main topics in the current psychology of individual difference: intelligence, genius and their evil counterpart, mental retardation; educational achievement; personality; psychopathology and criminality. Systematically he tackles three basic questions in turn: what is the conceptual status of the difference(s) involved; how far differences are genetically determined; and how far they are environmentally conditioned. At each point the extensive survey of evidence and research is made more valuable because of the careful and critical attention devoted to methodological aspects in the studies reviewed. There is a similarly critical and thorough treatment in the final section which is concerned with three types of group difference: those based on sex, age and race.

In any general text there are bound to be omissions. Here the discussion of occupational and career differences is narrow in scope, excluding a whole range of studies into attitude and performance. There is no reference to the main body of Kohlberg's research into moral differences, or to the investigation of differences in values. The assessment of variety in the forms of excellence is also limited: the work of Piaget, for instance, occupies but two pages, and interesting research by Hudson and others on divergent thinking is altogether ignored. It is perhaps surprising, too, that in the section on group differences there is no separate chapter on social class, and the implications of Bernstein's enquiries into language do not receive appropriate attention in the relatively casual account of conclusions reached by Hess and Shipman. Nevertheless, this book generally provides a scholarly and dispassionate review of a massive research field, and does so succinctly and clearly. It may be recommended to the intending student and teacher of psychology, and also offers the interested lay reader material that is both readable and substantial.

The companion volume of readings is well selected and appropriate to the text. Contents have been skillfully edited to limit the inclusion of spurious material and, in company with the text, they serve to illustrate both the conclusions of research and some of the inherent methodological preoccupations. **John A. Glossop**

John A. Glossop is Lecturer in Education in the School of Education, University of Leeds, UK.

obituary

Arend Bouhuys

AREND BOUHUYS died on board the *Queen Elizabeth 2*, of a sudden and unexpected heart attack, on 15 June 1979, at the early age of 53. He had resigned from his appointment at Yale University and was on his way to Europe to become chairman and professor of the Department of Physiology at the University of Utrecht in his native country.

His untimely death has deprived occupational medicine and respiratory physiology of an active and brilliant research worker who had made an outstanding contribution to environmental lung disease and, in particular, byssinosis or brown-lung disease suffered by textile workers.

Bouhuys was born in Deventer, in The Netherlands, in 1925. He graduated MD at Utrecht in 1948. He spent his early years as a resident in internal medicine and chest diseases and as an instructor in physiology. He soon developed a special interest in pulmonary disease and methods of measuring lung function. At the age of 29 he was appointed head of the Pulmonary Function Laboratory at the University of Amsterdam. He had by then become interested in byssinosis and in 1959 he published the first of a series of original papers on effects of cotton, flax, and hemp dust exposure on respiratory symptoms and lung function.

In 1962 he left The Netherlands to take up an appointment in Emory University School of Medicine in Atlanta, Georgia. Two years later he was made a Fellow of the John B. Pierce Foundation in New Haven and was offered an associate professorship at Yale University where, in 1968, he was appointed full Professor of Medicine and Epidemiology. During his 15 years at Yale his output was prolific. He published 86 original research papers, two books on the physiology of breathing and lung disease and numerous review articles and abstracts. In 1975 he became managing editor of *Lung* which, under his direction, attracted good papers and increased its circulation.

By using computers for recording and analysing respiratory symptoms (by questionnaires) and lung function, he developed sensitive and accurate methods of measuring acute and permanent changes in lung function from exposure to air pollutants. In this way he advanced epidemiological techniques for investigating chronic respiratory disease in communities. His most ambitious study, undertaken by a multidisciplinary team, was of 8,000 community residents in urban and rural districts in Connecticut and a rural population in South Carolina.

Two of his most valuable papers were published a year before he died. One gave new equations based on height, age and weight for predicting normal lung function values in healthy blacks and whites. The other paper was published in *Nature* **276**, 466-471, (1978). It produced evidence from his own and other studies that cigarette smoking and textile dust exposure were far more deleterious to health than out-door air pollution. He concluded that no measurable effects could be expected from further air pollution control with respect to prevalence of chronic bronchitis, asthma and loss of lung function, but, instead, there should be systematic efforts to prevent cigarette smoking and to control exposures to textile dusts and other inhalant risks at work.

In Utrecht he planned to continue research into isolating the causal agent of byssinosis.

If Arend Bouhuys had not been strongly motivated to apply his considerable skills and energy to the prevention of lung disease, he would have remained a laboratory research worker. Instead, with his co-research workers in various parts of the world, he undertook field studies of textile workers in several countries, including Spain, Holland and the United States. In the U.S. he focussed attention on brown-lung disease, which had been overlooked. His research findings and his own personal commitment helped to establish strict occupational health requirements for US cotton workers through dust control and medical surveillance.

He is survived by his wife, Fenna, who helped him a great deal in his work, particularly in preparing papers; a son and four daughters, two by a former marriage.

R.S.F. Schilling

P.C. Caldwell

THE DEATH of Professor P.C. Caldwell FRS, on 6 June 1979 at the age of 52 saddened his many friends and came while he was still at the height of his powers.

He was born in Warrington and belonged to a family which had lived there for several hundred years and prospered despite suffering heavy penalties for its steadfastness in the Roman Catholic faith. He was sent to Ampleforth School and there his exceptional academic gifts were recognised but these were so varied that the choice of career proved difficult. He finally decided on a scientific career and went as a scholar to Trinity College, Oxford where he studied physical chemistry for his first degree.

After graduating he stayed in Oxford to do research with Sir Cyril Hinshelwood on nucleic acid in bacteria. Together they showed that DNA per cell mass was related to the rate of growth, and hence protein synthesis, and discussed the need for a relationship between nucleic acid and protein, suggesting that two nucleotides would specify one amino acid. This was the first discussion of the coding problem.

From Oxford Peter Caldwell went to work with Professor A.V. Hill and Sir Bernard Katz at University College, London. It was there that he began his main life's work on the application of physico-chemical methods to biological problems. He made the first successful experiments in which micro-electrodes were used to measure intracellular pH. This led to a whole field of study in which similar electrodes are used to measure intracellular ion activities *in vivo*. In the course of this work he made the useful discovery that the leg muscles of the crab *Maia squinado* consisted of very large fibres 1-2 mm in diameter.

A.V. Hill believed very strongly that physicists and chemists entering biophysics should be sent to a marine biological station and so exposed to a great range of biological material and problems. It was not surprising, therefore, that Caldwell moved next to the Marine Biological Association's Laboratory in Plymouth and there, and at Bristol University, he continued to make key experiments in difficult and very competitive fields of study, often inventing powerful new techniques. With Sir Alan Hodgkin, Professor R.D. Keynes and other colleagues he played a leading role in the spectacular post-war advances in our knowledge of nerve and muscle.

Among the results of his many studies were: the first determinations of ATP, arginine phosphate and orthophosphate in single cells; providing the first example of a spatially orientated enzyme in a living membrane showing different sensitivities to inhibition on its two sides; and finding, on single crab muscle fibres, the first evidence that *ionised* calcium exists in very low concentrations in cells and that cell activity can be triggered by an increase in this calcium level. At Bristol University he trained a series of postgraduates in research and, with them, made important progress in our knowledge of the influx and efflux of substances from living cells. He was elected FRS in 1975.

Caldwell had many interests to which he gave himself wholeheartedly and in all of these his knowledge was deep and his technical skill so high that he could deal

with acknowledged experts on equal terms. An excellent pianist, he was devoted to music and composed about 30 piano sonatas. It was in accord with his character that he was especially attracted by the work of composers who, like Schubert, took a theme and followed it through many variations.

Peter Caldwell's exceptionally gentle and kind personality made him much appreciated by a wide circle of friends. When relaxed he was most entertaining company and he was always helpful in the lives and work of his colleagues and students. He will be widely missed and our deepest sympathy goes to Mrs P.A. Caldwell and their children Helen, Margaret, Philip, Anne and Thomas.

E.J. Denton

John Jaeger

PROFESSOR JOHN CONRAD JAEGER, Emeritus Professor of Geophysics in the Australian National University, died on 15 May 1979 after a long illness, at the age of 71.

Born in Sydney on 30 July 1907, he was educated at the Church of England Grammar School, Sydney, and at the University of Sydney from which he graduated Bachelor of Science in 1928 with first class honours and university medals in mathematics and physics. In that year he went to Cambridge University where he received first class honours in the Mathematical Tripos. Following five years of research at Cambridge in theoretical physics, he joined the University of Tasmania in 1936 as Lecturer in Mathematics, subsequently becoming Professor of Applied Mathematics in that University. In 1942 he was awarded the degree of Doctor of Science in the University of Sydney.

Shortly after the founding of the Australian National University, John Jaeger was invited to accept a Chair and to set up a Department in the Research School of Physical Sciences. He took up his appointment as Professor and Head of the Department of Geophysics in 1952, and remained in that post until his retirement in 1972. Under his direction, the Department of Geophysics developed rapidly into one of the leading research groups of its kind in the world. In 1965, it became the Department of Geophysics and Geochemistry and more recently (1973), was reconstituted to form the Research School of Earth Sciences, at least in part in recognition of the high international reputation that the department established under John Jaeger's leadership.

The direction of development of the department was largely based upon his strongly held belief that the application of the basic sciences of mathematics, physics and chemistry, in concert with more conventional geological methods, to the study of the earth, would result in major

advances in our understanding of the origin and history of the earth. His recognition of the importance of interdisciplinary fields, together with his ability to attract staff and students, were major factors in establishment of the high reputation of the department, both nationally and internationally, in a relatively short period.

John Jaeger had a most distinguished career as mathematician, physicist and geophysicist. His scientific contributions covered an extraordinarily diverse range of interests, including theoretical physics, radiophysics, ionospheric and solar physics and meteorology, but the main emphasis of his research was in the application of mathematical techniques to a wide variety of theoretical and practical problems, especially the conduction of heat. On establishing the Department of Geophysics in the Australian National University he embarked upon vigorous experimental programmes concerned with heat flow in the Australian continent, and the behaviour of rocks under stress (rock mechanics). John Jaeger was a man of great scholastic achievement; he published more than 130 articles in the scientific literature, and was author or coauthor of six books in applied mathematics and rock mechanics, which are regarded as standard works in their fields. His book (with Carslaw) *The Conduction of Heat in Solids* is widely accepted as a classic in this area.

In recognition of his achievements, Jaeger, in 1954, was elected a Fellow of the Australian Academy of Science, of which body he was Vice-President in 1958-1959, and in 1970 he was elected Fellow of the Royal Society (London). He was also Doctor of Science (*honoris causa*) of the University of Tasmania. In 1971, he was awarded the Rankine Lecture by the Institution of Civil Engineers (London).

John Jaeger was a big man, both physically and mentally. He was a man of great humanity but basically very shy. He never sought the limelight and was extremely modest about his own achievements. He set high standards for himself and expected staff and students alike to strive for excellence. His influence in many fields, including the earth sciences, in Australia and indeed in the world, has been profound. He will be sorely missed.

Ian McDougall

J.B. Birks

DR JOHN B. BIRKS, reader in physics at Manchester University died suddenly on 1 March 1979, a few months after the death of his wife Margaret in a car accident. He leaves a son and daughter.

He was born on 6 March 1920. After graduation from Oxford in 1940 he joined the Telecommunications Research Establishment for the duration of the war. His work there on radar led to his work on

microwave absorption in ferrites, when he joined the Department of Natural Philosophy at Glasgow and for which he received his PhD in 1947. An interest in scintillation counters led him into the field of scintillators and the photophysics of organic molecules which became his main scientific interest.

In 1951 he became professor of physics at Rhodes University, South Africa, where he quickly established an active research group. However, his dislike of the developing racial policies made him return to Britain in 1954 as director of research at British Dielectric Research. In 1957 he moved to Manchester to resume his work on photophysics and in a few years had built up a large and thriving research group which later, with his encouragement, diversified into electron scattering, lasers, microwave spectroscopy, vacuum ultraviolet spectroscopy and latterly, polymer physics.

An empiricist, he was probably most gifted in correlating the mass of data in the field and extracting the essence. He had a good physical insight and made substantial contributions to his subject. A prolific and fluent writer he published some 180 papers, edited the proceedings of many conferences, e.g. the Rutherford Jubilee International Conference 1961, and the series *Progress in Dielectrics*. He is probably best known for his books *Scintillation Counters* (1953), *Theory and Practice of Scintillation Counting* (1964), *Photophysics of Organic Molecules* (1970) and the two volumes of *Organic Molecular Photophysics* (1974). Several monographs on scintillation counting were widely distributed by several companies in the field. He was a consultant to many companies and national laboratories, in demand as a seminar speaker and a great attendee of conferences where he met his many friends who will now sadly miss his stimulating presence. An enthusiast for his subject he was on occasions guilty of going a bit over the top but his friends would readily forgive him.

John Birks was a man of wide culture and liberal humanistic views, and was always prepared to defend what he thought was right and to support his friends. Never an establishment man he clashed with authority on several occasions which possibly affected his career. A most efficient and capable administrator, and an excellent teacher and researcher he had much to offer.

He was a generous host who entertained well and was particularly kind to his students, many of whom came from overseas. He made them feel very much at home, and now, spread around the world, they will be saddened by his death.

The loss of his wife was a great blow. They will both be greatly missed by his son and daughter and their families. I am glad that for 28 years I knew him both as friend and colleague.

Scott Hamilton